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11012

Effect of X-rays on a Tumor of Known Genetic Constitution.*

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In a recent preliminary report[1] attention was called to an apparent genetic change produced experimentally, in a transplantable mouse cancer. As outlined in the previous paper, tumor dbrB of known

* Aided by a grant from the International Cancer Research Foundation. Read before the 32nd annual meeting of the American Society for Cancer Research, Richmond, Virginia, April 5th and 6th, 1939.

1 Reinhard, M. C., and Warner, S. G., to be published in *Radiology*.

genetic constitution, when transplanted into mice of the dbr strain, would grow in practically 100% of the animals. On the other hand, when transplanted into any one of several other pure strains of mice, namely, Little's C-57 Blacks, Strong's CBA and a strain of albinos known as the A stock, there was complete failure of tumor growth. This tumor, however, when treated with X-rays responded in an entirely different manner, in that comparatively small doses of X-rays produced some change in the tumor which caused it to grow when transplanted in approximately 40% of the animals of the various strains just mentioned, yet did not affect the percentage of takes in the dbr strain. These results lead us to draw the tentative conclusion that the X-rays produced a change in the genetic constitution of the tumor. However, this conclusion was based on a total of only 46 animals of the 3 resistant strains and results from a greater number of animals are necessary to make the data significant.

The present paper brings the status of these experiments to date. In addition to the use of a greater number of animals, the experiment has been amplified to include the following 3 considerations:

1. The continuance of the radiated dbrB tumor into successive transplant generations.
2. The effect of variation of dosage on the dbrB tumor.
3. The effect of radiation on another transplantable tumor (New Buffalo tumor).

The history of the dbrB tumor and the experimental procedure followed were given in detail in the preliminary report. However, a brief summary is included here. Tumor dbrB is a transplantable tumor of known genetic constitution. It originated as a spontaneous adenocarcinoma in 1920, in the dilute brown stock of mice. This tumor has been maintained successfully by repeated transplantations for approximately 19 years. During this period the tumor has been tested repeatedly and its genetic constitution determined from time to time. The results of these tests show that the tumor dbrB has a genetic factor ratio of 3:1.

Tumor fragments implanted into the dbr strain grew to a size of approximately one cubic centimeter over a period of 7 to 10 days. At this time the tumors were radiated, the body of the mouse being covered with lead in which a window was cut so as to permit the X-rays to strike the tumors. The distance, rate and quality of the radiation was the same as reported previously, 30 cm, 63.3 r/minute and λ effective 0.16 Å respectively.

Doses of the order of 1500 r caused regression in the original host. The results of radiating dbrB tumors with 100 r and subsequently

TABLE I.
Results of Transplanting dbrB Tumors.*

Stock	Total	No. positive	No. negative	% positive
		Dose 100r.		
dbr	111	110	1	99.09
C-57 Black	105	48	57	45.71
CBA	60	23	37	38.33
A Stock	81	31	50	38.27
		Dose 50r.		
C-57 Black	57	23	34	40.33

* In order to prove that tumor fragments of dbrB will grow in the dbr strain only, 104 CBA's, 97 C-57 Blacks and 93 of the A stock were inoculated with normal dbrB tumor. There were no takes in any of these mice.

transplanting them into 111 mice of the susceptible strain (dbr) and into 246 mice of the 3 resistant strains are shown in Table I.

In addition to the 100 r dose several dbrB tumors were subjected to a dose of 50 r given also *in vivo*. As shown in Table I there was approximately the same percentage of takes in 57 animals of one of the 3 resistant strains (C-57 Black). This lends proof that small doses of the order of 50 to 100 r are capable of producing genetic changes in this particular tumor.

If this change in the tumor produced by these small doses of fairly hard X-rays is constitutional, we would expect additional transplantation into succeeding generations to yield approximately the same number of takes. Successive transplants of the radiated tumor through the fourth transplant generation to date (Table II) have yielded approximately 40% takes, adding further proof to the contention that a genetic change has been produced.

When the radiated tumor dbrB was implanted into mice of the dbr strain, there were practically 100% takes. This was true also of the control experiment, in which non-radiated tumor fragments grew in approximately 100% of the mice of the dbr strain. Simultaneous transplantation of the non-radiated tumor into the 3 resistant strains (C-57 Black, 41; CBA, 37; A, 43) resulted in no takes, indicating that no spontaneous change had occurred in the tumor during the course of the experiment.

Since we have presumably produced genetic changes with X-rays

TABLE II.
Results of Successive Transplantations of Radiated dbrB Tumor into C-57 Black.

Generation	Total	No. positive	No. negative	% positive
First	56	21	35	37.50
Second	39	16	23	41.02
Third	63	27	31	42.85
Fourth	32	12	20	37.50

in one group of tumors, (dbrB tumor) the question may well be raised, would similar doses produce a comparable change in another group of tumors of different origin. Therefore a second group of tumors (New Buffalo tumor) was selected for additional experimental work following the same procedure as that used for the dbrB group of tumors. The New Buffalo tumor also originated as a spontaneous adenocarcinoma and has been successfully propagated for several years by transplantation in a pure strain of albino mice known as the New Buffalo Strain, where practically 100% takes are obtained. This tumor will not grow in other pure strains, C-57 Blacks, CBA's.

To date we have exposed these tumors to a 100 r dose of X-rays and have transplanted them into only one of the resistant strains, C-57 Blacks. As shown in Table III there were takes in 37% of 51 animals, approximately the same percentage of takes in this particular host strain as was obtained with tumor dbrB. Additional transplantation into the other resistant strains is underway.

TABLE III.
Results of Transplanting New Buffalo Tumor.
Dose 100r.

Stock	Total	No. positive	No. negative	% positive
New Buffalo	51	19	32	37.25

Conclusions. The data presented in this paper involving, (1) the use of approximately 300 animals, (2) 2 different transplantable tumors known as dbrB and New Buffalo Tumor, (3) the maintenance of the radiated tumor through the fourth transplant generation, are indicative of the fact that small doses of X-radiation are capable of producing a change in the genetic constitution of a tumor.

It is interesting to note in this connection that while X-rays have been used to produce mutative changes in certain forms of life, such as plants, insects and animals, the dosage required for these changes is far in excess of that which we used to produce changes in the constitution of the tumors.

As shown by Muller and others, thousands of roentgens are needed to produce mutations and the higher the dosage the greater is the mutational effect. In our experience we have been unable to distinguish any quantitative difference between doses of 50 r and 100 r. Doses of 1500 r cause regression.

11013 P

The Biologic Nature of Sulfapyridine's Bacteriostatic Effect Against the Pneumococcus.

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This paper represents an experimental attempt to show that the morphologic alterations characteristically induced by the sulfonamide compounds against the pneumococcus are of the nature of a phasic dissociative change rather than "involutionary", in the monomorphic sense. This in accordance with our position as previously outlined at the June, 1939, meeting in Milwaukee of the American Association for the Advancement of Science. (In press.)

Two series of mice were employed: the first of 20 animals, and the second of 10, with 10 controls for each. The animals were infected intraäbdominally with 0.5×10^{-6} cc of a 16-hour veal-infusion broth-culture of Type II pneumococcus (Binda strain). Therapeusis was started in 2 hours and consisted of 4 doses daily at 4-hour intervals, of 0.25 to 0.5 cc of a 1:4 dilution of a sulfonamide carbohydrate compound.* Cultures and smears of peritoneal exudate were made at alternate treatments between the 3d and 15th.

After 16 hours the first response was obtained. It consisted of a marked clumping of the organisms both about the cellular elements and independently. After 20 hours, the free organisms in the smears showed capsules so swollen as to resemble a Neufeld reaction. There was also a definite phagocytosis of the swollen organisms by the polynuclear and macrophagic cells, whose margins were no longer clear-cut. Adherent to them were microörganisms and a granular material, possibly fibrin.

At this time the mucoid colonies were slightly umbilicated and microscopically contained a pleomorphic phase that, while not isolatable at this time, at the 40-hour period dissociated as a dwarf colony of non-encapsulated pneumococci reacting negatively to the Neufeld-test. It was bile-soluble, fermented inulin slowly, and morphologically the cocci were elongated to a point where they often resembled diphtheroids; or they formed long chains. Their methemoglobin-

* In order to increase its solubility the sulfapyridine is dissolved in galactose and nucleic acid. To this mixture is added some sulfanilamide that has been combined with xylose, which completes the solubility of the sulfapyridine. Dr. Fritz Meyer and his associates of New York are responsible for this preparation and we are indebted to him for the samples used in this study.

formation was greatly diminished. The "dwarf" strain was further dissociated into a minute or G-type colony (Hadley) whose morphology was that of a micrococcus instead of a diplococcus. Both the "dwarf" colony and the G-type were avirulent.

After the 7th and 9th treatments phagocytosis of the encapsulated organisms markedly increased and the number of free organisms diminished. Their clumping, both free and about the cells, was still evident. After the 11th treatment there was suggestive evidence of lysis of the organisms. This was more marked after the 15th treatment when the cultures were sterile.

The morphology of the encapsulated pleomorphic forms in the umbilicated mucoid colonies is so similar to that of the Neufeld-negative dwarf colonies that we regard the latter as the direct descendants of this pleomorphic phase. In fact, the latter which was diphtheroid-like in morphology, has now been isolated as an independent entity. Moreover, the pleomorphic forms and diplococci, both with swollen capsules, are apparently phagocytable even before their colonial dissociation as a phasic entity occurs. The relative ease with which Neufeld-negative colonies revert to the virulent mucoid phase,[1] together with their other characters, suggests that they fall into a different category of variational change from the M, S, and R pattern; although the fact that Hadley has recently shown that similar changes with the hemolytic streptococcus *in vitro* can carry through to a typical S-colony form, makes it appear that the two categories are essentially the same in nature. In other words, the pleomorphic diphtheroidal forms of the mucoid culture, at a time when they are not yet dissociable, have a *true phasic status* by virtue first, of their phagocytability, and second by a diminution of their principal metabolic activities, such as methemoglobin formation and bile solubility.

The early *in vivo* phagocytability of these pleomorphic phases which thus presages their early destruction, probably explains the clinical and experimental infrequency[1, 2] of the Neufeld-negative or other non-virulent dissociant such as the S-colony. We are inclined to regard the capsular change merely as a part of the dissociative picture rather than a specific effect of the drug on this structure *per se*.

It would appear then, that the bacteriostatic action of the drug *in vivo* is essentially of the nature of a phase-transformation, which brings the germ within killing range of the immuno-phagocytic defenses of the host. This point of view is in accord with the necessity for the administration of repeated small doses, whose effect both *in vivo* and *in vitro* is dominantly dissociational rather than bactericidal.

1 Hilles, C., and Schmidt, L. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 73.
2 Telling, M., and Oliver, W. A., *Lancet*, 1938, 234.

These phasic changes are regarded as being implemented chiefly, but not necessarily solely, by the accumulation of hydrogen peroxide, which may be accounted for on the basis of our demonstration of the anticatalase-action of the sulfonamide compounds.[3-6]

11014

Choline Esterase Activity in Blood Serum and Duodenum of Beriberi Pigeons.

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It has been recently demonstrated by Glick and Antopol[1] and by Süllmann and Birkhäuser[2] that thiamine exerts an inhibitory action upon choline esterase *in vitro*. Zeller, Schär and Staehlin[3] showed that the splitting of histamine and diamines by the diamino-oxidase is also inhibited by thiamine. It has been shown by Goodhart and Sinclair[4] that cocarboxylase is contained in the cellular elements of the blood and the free thiamine essentially in the plasma. In contrast to thiamine, cocarboxylase recently has been found to have very little inhibitory effect upon the enzymes.[5] The question then arises whether thiamine has a physiological effect, *in vivo*, on choline esterase. To throw light on this point measurements were made of the activity of the enzyme in the serum of beriberi and normal pigeons. The concentration of the enzyme in the small intestine was also measured and correlated with its sensitivity to acetylcholine.

Twenty-two pigeons in 3 series were used in these investigations. In the first group, 6 pigeons were placed on a polished rice diet and choline esterase determinations were made about every 8 days on blood serum obtained from the wing vein. When the pigeon showed

3 Locke, A., Main, E., and Mellon, R. R., *Science*, 1938, **88**, 620.

4 Main, E., Shinn, L. E., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 272.

5 Shinn, L. E., Main, E., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 591.

6 Shinn, L. E., Main, E., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 640.

1 Glick, D., and Antopol, W., *J. Pharm. Exp. Therap.*, 1939, **65**, 389.

2 Süllmann, H., and Birkhäuser, H., *Schweiz. Med. Wchnschr.*, 1939, **69**, 688.

3 Zeller, E. A., Schär, B., and Staehlin, S., *Helv. Chim. Acta*, 1939, **22**, 837.

4 Goodhart, R. S., and Sinclair, H. M., *Biochem. J.*, 1939, **33**, 1099.

5 Glick, D., and Antopol, W., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 396.

nervous symptoms, the estimations were made at suitable shorter intervals.

In the second and third group, consisting of 6 and 10 pigeons each, the birds were first maintained on a diet consisting of oats, corn, and water for a period of 2 weeks in group 2, and 3 months in group 3. During this time repeated choline esterase determinations were made on the sera After an initial period of acclimatization, the enzyme concentration remained constant. Each group was then subdivided into 2 series. One of these was continued on the full diet while the other was transferred to one of polished rice. When polyneuritic symptoms were observed, measurements of choline esterase were again carried out. Parallel determinations were made on the normal group. At the height of the beriberi stage, the choline esterase determinations were made once again and the birds sacrificed by decapitation.

The duodenum was immediately removed for a distance of about 2 cm on each side of its first angulation. The distal portion was severed from the proximal, opened, and washed free of its contents with Ringer solution. Great care was taken to remove the bile, since it has been shown to inhibit the enzyme.[6, 7] The duodenal choline esterase was determined in the same manner as previously employed for other tissues.[8]

The proximal loop of duodenum was washed through with cold oxygen-saturated Locke's solution, and stored for 4 hours in the ice chest immersed in this solution. The sensitivity to acetylcholine was then determined by the Magnus method.

The serum choline esterase was determined by the Ammon method at 30°C as previously described[7] employing 0.5 cc of solution prepared by diluting the serum 10 times with bicarbonate-Ringer.

The results for all 3 groups were essentially the same, hence only the data for group III are given (Table I). From March 14 to May 17 the 10 pigeons were on a full diet and showed fairly constant choline esterase levels in the blood. On May 15, the 5 pigeons were placed on a polished rice diet while the others remained on a normal diet. It is seen from Table I that, whereas the choline esterase of pigeons on a normal diet remain fairly constant, those on a polished rice diet show an increased choline esterase with the development of polyneuritic symptoms.

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6 Sobotka, H., and Antopol, W., *Enzymologia*, 1937, **4**, 189.

7 Antopol, W., Schifrin, A., and Tuchman, L., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 363.

8 Glick, D., Lewin, A., and Antopol, W., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 28.

TABLE I.
Choline Esterase Activity of the Blood Serum of Normal and Beriberi Pigeons.

	Pigeon No.									
Date	15	16	17	18	19	20	21	22	23	24
2-19-39	All pigeons placed on normal diet									
3-14	144	170	138	135	120	146	129	165	128	190
3-16						163	152	170	144	186
3-26	162	158	137	134	122					
4-13	170	156	156	137						
4-17						117	111	160	125	183
5-12	158	154	159	141	147					
5-15						146	142	175	142	170
5-17	Remain on normal diet					Placed on polished rice diet				
6-12	167		166			200 (severe ataxia)		230 (severe ataxia)		
6-14		140		141	152				262 (severe ataxia)	240 (severe ataxia)
6-19						190* Opisthotonus				
6-20									225* (legs paralyzed)	200* (legs paralyzed)
6-22							223*			
6-23					155*					
6-27			130*							
6-28				129*						
6-29	137*									
6-30		140*								

* Killed.
Enzyme activity in blood serum expressed in mm^3 CO_2 liberated in 1 hr at 30° C.

Similarly, there is an increased choline esterase concentration in the small intestine of beriberi pigeons, as well as a decreased sensitivity of the small intestine to acetylcholine (Table II). The decreased sensitivity had been already observed by Abderhalden and Abderhalden.[9] In contrast to these findings, Beauvallet[10] reported no change in the acetylcholine sensitivity of the small intestine of pigeons when the birds developed polyneuritis from a diet consisting of proteins, lipoids, glucose, and minerals. Both Abderhalden and Abderhalden, and Beauvallet found that the addition of vitamin B_1 to the insensitive small intestine of beriberi pigeons increased its reactivity to acetylcholine. Mintz[11] showed that vitamin B_1 increases the activity of acetylcholine on the isolated intestine of the rat. Since thiamine inhibits choline esterase, the question arises whether the diminished sensitivity of the small intestine of the

9 Abderhalden, E., and Abderhalden, R., *Pflug. Arch.*, 1938, **240**, 388.
10 Beauvallet, M., *Compt. rend. soc. biol.*, 1938, **128**, 1020.
11 Agid, R., Beauvallet, M., and Mintz, B., *Compt. rend. soc. biol.*, 1937, **126**, 982.

TABLE II.
Enzyme Activity of the Duodenum and Its Sensitivity to Acetylcholine in Normal and Beriberi Pigeons.

Date	Normal pigeons No.	Enzyme activity	Acetylcholine concentration
6-23-39	19	46	1: 5,000,000
6-27	17	56	1: 5,000,000
6-28	18	52	1: 8,000,000
6-29	15	64	1: 5,000,000
6-30	16	42	1:10,000,000
	Beriberi Pigeons		
6-19	20	68*	1: 100,000
6-20	24	140	1: 100,000
6-20	23	134	1: 50,000
6-22	22	132	1: 1,000,000

Enzyme activity expressed in mm^3 CO_2 liberated in 1 hr at 30° C.
* Intestine not washed free of bile.

beriberi pigeon is due to the increased choline esterase, which, in turn, is dependent upon the absence of vitamin B_1 as has been previously suggested.[1] Our investigations confirm Abderhalden's findings of, first, a lack of spontaneous rhythmic intestinal contraction in beriberi pigeons, and, second, an increase in intestinal tone without production of rhythmic contractions after the addition of effective doses of acetylcholine. However, the small intestine of normal pigeons shows spontaneous rhythmic contractions and with acetylcholine there is first an increased tone and then an increase in intensity of the contractions. In addition, Abderhalden found that in the presence of thiamine, not only is tonus increased in the isolated intestine of beriberi pigeons, but rhythmic contractions occur. It appears, therefore, that vitamin B_1 is involved in the production of rhythmic contractions. The initiation of these rhythmic intestinal contractions may explain the findings of Frazier and Ravdin[12] that intestinal symptoms of hyperthyroid patients are alleviated by thiamine, since it was suggested that hyperthyroidism is associated with vitamin B_1 deficiency. This may be correlated with the observations of Antopol, Tuchman and Schifrin[13] that cases of untreated hyperthyroidism have relatively high choline esterase levels in the blood serum.

Summary. The choline esterase concentration was found to be increased in the serum and small intestine of beriberi pigeons. The small intestine of beriberi pigeons showed decreased sensitivity to

12 Frazier, W. D., and Ravdin, I. S., *Surgery*, 1938, **4**, 680.

13 Antopol, W., Tuchman, L., and Schifrin, A., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 46.

acetylcholine. The basis of these effects, and their significance has been discussed.

The authors wish to thank Miss Bessie Zirin, Mr. Sidney Morett and Mr. William Emich for their technical assistance in this investigation.

11015

Prevention and Modification of Measles with Concentrated Pooled Ascites Fluid and with Its Globulin Fraction.*

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Various types of antibodies have been demonstrated in ascites fluid, but the presence of measles-protecting substances has not been reported. Since measles-protecting substances are found in the blood serum of most urban dwelling adults, it was thought of interest to test for their presence in a number of ascites fluids. Should these antibodies be present, the large amount of ascites fluid available in hospitals could provide an additional source of material for measles prophylaxis.

The ascites fluid was obtained from Wassermann negative individuals suffering from portal cirrhosis or cardio-vascular-renal disease. After Wassermann, Kline and sterility tests were found negative, a pool was made of aliquot parts of 9 fluids. Since less protein is present in ascites fluid than in serum it was realized that this fluid would have to be concentrated to provide a dose small enough to be practicable for intramuscular injection. The ascites fluid pool was divided into 3 parts. One portion was concentrated in sterile cellophane sausage casings by the corn syrup technic previously described,[1] another portion by the air technic,[2] and the globulin fraction was prepared by 50% ammonium sulphate precipitation.[3]

To obtain an amount of measles-protecting substances in concentrated ascites fluid equivalent to that in measles convalescent

* This investigation was aided by a grant from the John and Mary R. Markle Foundation.

1 Thalhimer, W., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 230.

2 Thalhimer, W., PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 639.

3 Gibson, R. B., *J. Biol. Chem.*, 1906, **1**, 161.

serum or in pooled normal adult serum we calculated as follows: It has been found that about 4 volumes of pooled normal adult serum are necessary to give the same degree of passive immunity to measles as one volume of measles convalescent serum. Measles antibodies are contained in the globulin fraction of serum,[4, 5] as is true of other antibodies. Assuming that the globulin in ascites fluid is just as potent for measles prophylaxis as that in normal adult serum there would have to be as much globulin present in 1 cc of concentrated ascites fluid as in 4 cc of normal serum. Since normal serum contains 2% to 2.5% globulin, the concentrated ascites fluid would have to contain 4 times that amount, or 8% to 10% globulin.

The amount of globulin in the pooled unconcentrated ascites fluid was found to be 0.9%. The portion of the pool processed by the corn syrup technic was concentrated 9½ times, and was found by analysis to contain 9% globulin. The portion processed by the air technic was concentrated 5½ times, and analysis showed 5½ % globulin.† The other part was concentrated by globulin fractionation to one-tenth its original volume. If the separation of albumin and globulin had been complete this should have contained 9% globulin. However, analysis showed 7.2% globulin, a concentration of only 8 times or 20% loss of globulin, and, also, 1.4% albumin (Table I).

Since there is no laboratory method for determining the presence or amount of measles-protecting substances, we used a principle

TABLE I.

Ascites Fluid (Pool II), Concentrated by Two Cellophane Bag Methods and by Globulin Fractionation. Chemical Analyses and Assay of Diphtheria Antitoxin.

	Unconcentrated	Conc. by air method (5.5 times)	Conc. by corn syrup method (9.5 times)	Globulin fraction (Conc. 10 times)
Total Protein	2.4%	14. % (13.2%)†	25% (22.8%)†	8.6%
Albumin	1.5%	8.5% (8.25%)†	16% (14.25%)†	1.4%
Globulin*	0.9%	5.5% (4.95%)†	9% (8.55%)†	7.2% (9%)†
No. M.L.D.'s of Diph. Toxin neutralized by antitoxin in 1 cc	6	30-34 (36.6)‡	50-60 (60)‡	45-50 (48)‡

* Figures obtained by difference, after determining total protein and albumin.

† Figures in parentheses show the percentage calculated from chemical analysis of the original pooled ascites fluid on the basis of concentration.

‡ Figures in parentheses show diphtheria antitoxin calculated on the basis of the globulin content in the original pooled ascites fluid and globulin content of each concentrate.

4 Karelitz, S., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 793.

5 Karelitz, S., *Am. J. Dis. Child.*, 1938, **55**, 768.

† Because of the smaller amount of globulin in this concentrate prepared by the air technic a double dose was used.

introduced by Karelitz[6] of following the fate of another antibody, diphtheria antitoxin, in the original material and in the final preparations. The diphtheria antitoxin in the concentrated ascites fluids and in the globulin fraction increased in amount corresponding to the degree of the concentration of the globulin. Whereas the amount of diphtheria antitoxin is not an index of the amount of measles-protecting substances present, nevertheless this study demonstrated that the diphtheria antitoxin was not lost in these 3 methods of concentration if globulin was not lost, and it was expected that the measles-protecting substances, if present, would not be lost either.

Table I shows one typical pool, the degree of concentration, the percentage of total protein, albumin and globulin, and the amount of diphtheria antitoxin in the original pool and in the 3 concentrates.

The protective power of the 3 concentrates and of convalescent measles serum (used as a control) was determined clinically by administering them intramuscularly to healthy, susceptible children in crowded tenement houses where the contact had been intimately exposed to a member of the family developing measles. Under conditions of exposure of this type, about 90% of susceptible children will contract measles if unprotected. The dosage of the serums or concentrates was 10 cc, or a volume containing approximately an equivalent amount of globulin,† for all children regardless of age. The preparations were administered to all contacts from 4 to 7 days after exposure, considering the day the rash appeared in the patient as the 4th day after exposure. The contacts were observed daily or every other day from the 10th to 25th day after exposure. Rectal temperatures were taken on each visit. In this manner, mild forms of the modified disease, which might be overlooked, were detected, and the preparations were put to the severest test.

There were 23 children in the control group receiving regular measles convalescent serum; 24, receiving the globulin fraction of ascites fluid; 11, ascites fluid concentrated by the corn syrup technic, and 10, ascites fluid concentrated by the air technic. The results are shown in Table II.

That these preparations have a considerable ability to prevent and modify measles in the concentrations and quantities used, is shown by the fact that in all children where protection was not obtained the disease occurred in modified form, and not a single case of unmodified measles developed. In this series, though small, the measles prophylactic value of the ascites fluid, concentrated by the corn syrup and air technics respectively, appeared to be as good as and

6 Karelitz, S., Greenwald, C. K., and Klein, A. J., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1359.

TABLE II.

Results Obtained in Prevention and Modification of Measles with Concentrates of Ascites Fluid and Measles Convalescent Serum.

Measles Convalescent Serum Pool M-23 Dose 10 cc			Ascites Fluid-Globulin Fraction Conc. 10 times Dose 10 cc			Ascites Fluid Conc. by corn syrup technic 9½ times Dose 10 cc			Ascites Fluid Conc. by air technic 5½ times Dose 20 cc		
Age	Day(*)	Result*	Age	Day(*)	Result*	Age	Day(*)	Result*	Age	Day(*)	Result*
8 mo	6	O	12 mo	6	O	13 mo	4	O	2 yr	4	O
16 "	3	O	15 "	5	O	18 "	6	A	2 "	5	O
16 "	5, 4, 3	A	16 "	7	B	22 "	7	B	4 "	5	O
17 "	4	A	18 "	5	B	2 yr	5	O	4 "	5	O
18 "	5	O	2 yr	6	A	2 "	5	A	5 "	5	O
20 "	4	O	2 "	6	B	3 "	5	O	5 "	7	B
22 "	7	B	3 "	3	O	3 "	7	O	6 "	3	O
2 yr	3	O	3 "	4	A	4 "	5	B	6 "	5	O
2 "	5	A	3 "	5	A	4 "	7	C	7 "	5	C
2 "	5	O	4 "	4	B	6 "	7	O	12 "	5	O
2 "	6	O	4 "	4	B	11 "	5	O			
3 "	4	A	4 "	5	C						
3 "	5	B	4 "	6	O						
3 "	6	O	4 "	6	B						
3 "	6	O	5 "	3	O						
4 "	5	O	5 "	5	O						
4 "	6	B	5 "	6	C						
5 "	4	A	6 "	5	C						
5 "	5	C	6 "	7	A						
5 "	5	B	6 "	7	O						
5 "	6	O	7 "	6	O						
7 "	7	O	8 "	4	O						
11 "	8	B	9 "	6, 5	B						
			12 "	8	B						
Results:		%			%			%			%
O	12	(52.2)	O	9	(37.5)	O	6	(54.5)	O	8	(80)
A	5	(21.7)	A	4	(16.6)	A	2	(18.2)			
B	5	(21.7)	B	8	(33.3)	B	2	(18.2)	B	1	(10)
C	1	(4.4)	C	3	(12.6)	C	1	(9.1)	C	1	(10)
	23			24			11			10	

* O—No disease developed. A—Extremely mild, barely recognizable modified measles. B—Mild, modified measles. C—Modified measles, definitely less severe than unmodified measles.

(*) Day of exposure on which the contact received the preparation.

better than that of the measles convalescent serum, while the globulin fraction in the concentration used was about three-fourths as efficient (Table II). The efficiency of these concentrates was proportional to their globulin content, the best results being obtained with the ascites fluid concentrated by the air technic, which contained 1.1 g of globulin in the 20 cc dose used. The next best results were obtained with the ascites fluid concentrated by the corn syrup technic, which contained 0.9 g of globulin in the 10 cc used. The least favorable results were obtained with the globulin fraction which contained 0.72 g per 10 cc. By increasing the amount or concentration of the globulin fraction results quite comparable to those obtained with measles convalescent serum and the ascites fluid concentrates should be obtained.

No local or general reactions occurred following the intramuscular injection of 10 cc of convalescent measles serum. Injection of the other solutions produced slight transitory local pain and stiffness in a small number of the children, but the temperature was seldom elevated above 100°. The injections of 20 cc amounts produced more local discomfort than the 10 cc doses.

In hospitals and private practice we have found that regular measles convalescent serum gives a higher percentage of protection than was obtained in this investigation. This is what one would expect where the exposure of contacts is less intimate and less prolonged, and the hygienic surroundings are better, and is in accord with the reported observations of others.[7, 8]

Summary. Measles-protecting substances have been found in pooled ascites fluid and its globulin fraction in sufficient quantities to be of practical value. The efficiency in measles prophylaxis of 2 concentrates of pooled ascites fluid and of a globulin fraction was found proportional to the globulin content of each preparation.

Acknowledgments: Sophronia A. Myron and Thelma Schwartz prepared the ascites fluid concentrates. The globulin fraction was prepared by Mr. E. Cardone under the direction of Dr. K. G. Falk in the chemical division of the Research Laboratory of the New York City Department of Health. The diphtheria antitoxin was determined by Mr. C. Greenwald in the same laboratory. Dr. Samuel Frant, Epidemiologist of the New York City Department of Health, has been very helpful and cooperative.

For supplying us with large amounts of ascites fluid we are especially indebted to Dr. A. J. Patek, Jr., Metropolitan Hospital; Dr. William Tillett, Bellevue Hospital; Dr. Samuel Rosen, Montefiore Hospital; Dr. J. H. Cudmore and Dr. C. Royster, City Hospital; Dr. E. J. Ryan, St. Luke's Hospital; Dr. B. S. Oppenheimer, Mt. Sinai Hospital; and Dr. E. Sanford, Roosevelt Hospital.

7 Park, W. H., and Freeman, R. G., Jr., *J. A. M. A.*, 1926, **87**, 556.

8 Karelitz, S., and Schick, B., *J. A. M. A.*, 1935, **104**, 991.

11016 P

Existence of an Endocrine Gland in the Media of the Renal Arterioles.

N. Goormaghtigh. (Introduced by A. Brunschwig.)

From the Laboratory of Pathology, University of Ghent, Belgium.

The juxta glomerular apparatus is composed mainly of smooth muscle cells devoid of myofibrils called afibrillar cells (abbr: a. f. cells).[2,4] In the superficial cortical zone of the normal kidney of the rabbit, these cell-groups show a glandular cycle culminating in the formation of acidophil or basophil secretion granules intermingled with minute vacuoles (Bouin-Hollande or Zenker-formol fixation). They are in close contact with the lumen of the vas afferens or with capillaries. The endocrine features of these cells are as definite as those of the chromophil cells of the anterior pituitary. Three weeks of moderate constriction of the left renal artery of young rabbits (Drury technic[1]) which causes the shrinkage of a few glomeruli, are followed by an increase in number and size of the granulated cells in the superficial juxta glomerular apparatuses. (R. 31-91; 31-92; 31-93.) After the same lapse of time a more pronounced constriction causes the hyalinization of the superficial glomeruli and the regression of many tubuli, but leaves the renal arterioles patent. (R. 31-96; 31-97; 31-97). In these kidneys the increase in number of the granulated, a. f. cells is remarkable. Not only do they exist at the vascular pole of the intact or hyalinized glomeruli but they invade the latter and become conspicuous in the wall of all the arterioles of the cortex; a considerable number of smooth muscle cells still spindle-shaped, change into granulated a. f. cells. The granules become basophil. (Masson's trichrome technic.)

Identical changes occur in the ischemic kidney of the dog, but are not so readily recognized because the a. f. cells do not contain secretion granules normally. From a renewed survey of the ischemic kidneys of the 12 dogs referred to in a previous communication[3] the behavior of the a. f. cells in acute or subacute experiments can be summarized as follows: hypertrophy, hyperplasia and vacuolation of the a. f. cells of the juxta glomerular apparatus; occasional pro-

[1] Drury, D. R., *J. Exp. Med.*, 1938, **68**, 693.

[2] Goormaghtigh, N., *J. Physiol.*, 1937, **90**, 1263.

[3] Goormaghtigh, N., and Grimson, K., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 227.

[4] Goormaghtigh, N., and Handovsky, H., *Arch. Path.*, 1938, **26**, 1144.

trusion of the a. f. cells in the glomerular tuft; hyperplasia of the a. f. cells of the vas afferens followed by glomerular regression; transformation of ordinary smooth muscle cells into a. f. cells: a process which is accompanied by mitotic activity. In chronic cases of eight and seventeen months 'duration all the juxta glomerular apparatuses are hyperplastic and secretion granules appear in the a. f. cells while no qualitative changes occur in the tubules.

Since renal ischemia causes hyperplasia and hypertrophy of the a. f. cells which in the control rabbit have cytologic features of endocrine activity; since carefully graduated ischemia stimulates the a. f. cells at the exclusion of any qualitative changes in the tubules and favors the apparition of secretion granules in the a. f. cells of the dog, normally devoid of them, it must be concluded that the endocrine activity of the a. f. cells is related to the production of the hypertensive substance present in the ischemic kidney. It is suggested that in normal conditions the a. f. cells regulate the tonus of the renal arterioles.

11017

Effect of Added Glucose on Rate of Appearance of Free Sugar in Liver Brei.

S. SOSKIN, R. LEVINE AND M. TAUBENHAUS.

From the Department of Metabolism and Endocrinology, Michael Reese Hospital, and the Department of Physiology, University of Chicago.

Previous work has shown that a rise in the blood sugar level in the normal animal causes a compensatory decrease in the sugar output of the liver.[1, 2] The operation of this homeostatic mechanism to maintain the normal blood sugar level depends upon the presence of the equal and opposite influences of insulin and other hormones. But, providing this endocrine balance is normal, no extra insulin need be secreted for each regulatory action.[1] Indeed, the regulation has been demonstrated in the "Houssay animal," in which insulin and the anterior pituitary hormones are entirely lacking.[3] The ab-

1 Soskin, S., Allweiss, M. D., and Cohn, D. J., *Am. J. Physiol.*, 1934, **109**, 155.

2 Soskin, S., Essex, H. E., Herrick, J. F., and Mann, F. C., *Am. J. Physiol.*, 1938, **124**, 558.

3 Soskin, S., Mirsky, I. A., Zimmerman, L. M., and Heller, R. C., *Am. J. Physiol.*, 1936, **114**, 648.

sence of the hormones representing both sides of the endocrine balance does not cause as great a disturbance as when one side is allowed to act unopposed, and results merely in an upward shift of the blood sugar level (threshold) at which regulation occurs.

These results indicated the existence of an intrinsic regulating mechanism in the liver cells, dependent upon the endocrines only for the fine adjustment of its threshold of response. The present work was done with minced liver *in vitro* to confirm the postulated intrinsic hepatic mechanism, by eliminating all extra-hepatic factors.

Livers of normal, fed dogs were used for all experiments. The organ was removed under nembutal anesthesia, and finely minced in a previously cooled meat grinder. Samples weighing approximately 1.5 g were put into Erlenmeyer flasks containing 5 cc of Hastings' phosphate solution.[4] The flasks were oxygenated for 5-10 minutes, and shaken in a water bath at 38°C.

Total carbohydrate and free sugar were estimated by the method of Tsai[5] as modified by Benoy and Elliott,[6] using the Somogyi reagent. Inorganic phosphate was determined by the method of Fiske and Subbarow,[7] as adapted to the photoelectric colorimeter.

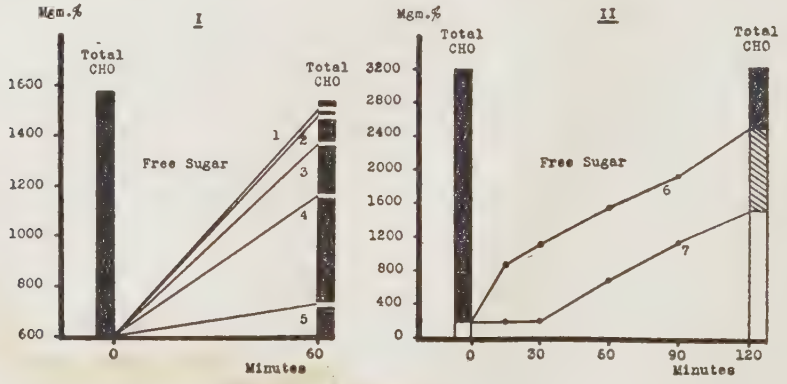

FIG. 1.

I. The influence of different amounts of glucose added to each vessel, upon the appearance of free sugar in liver brei in 1 hour. 1. No addition. 2. 5 mg glucose added. 3. 10 mg added. 4. 20 mg added. 5. 40 mg added.

II. A comparison of the rates of appearance of free sugar at different time intervals, with and without the addition of glucose to liver brei. 6. No addition. 7. 20 mg glucose added.

The blocks representing total carbohydrate determinations at the beginning and end of each experiment, indicate that there was no significant loss of carbohydrate from the system.

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4 Hastings, A. B., Muus, J., and Bessey, O., *J. Biol. Chem.*, 1939, **129**, 295.

5 Tsai, L., *Chinese J. Physiol.*, 1933, **1**, 91.

6 Benoy, M. P., and Elliott, K. A. C., *Biochem. J.*, 1937, **31**, 1268.

7 Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, **66**, 375.

TABLE I.

Inhibition, by added glucose, of appearance of free Sugar in mg per 100 g of Liver, in 60 Minutes.

Amt of dextrose added mg %	Exp. No. 1			Exp. No. 2			Exp. No. 3		
	Free sugar			Free sugar			Free sugar		
	Min 0	Min 60	% Inhib.	Min. 0	Min. 60	% Inhib.	Min. 0	Min. 60	% Inhib.
0	254	1997	—	184	745	—	172	301	—
100	”	2027	0	”	567	24	”	224	25
200	”	1565	22	”	585	22	”	174	42
400	”	1229	38	”	330	55	”	151	50
800	”	1260	37	”	316	58	”	208	31
1600	”	958	52	”	376	50	”	—	—
Total CHO	3313	3578	—	1234	1209	—	404	401	—

Representative results are shown in Fig. 1 and Table I. It can be seen from these data that the addition of dextrose at the beginning of the incubation period inhibits the rate of appearance of free sugar for ½-1 hour, after which the rate of appearance of free sugar begins to approximate that of the control (without added sugar). The degree of inhibition is, within certain ranges, proportional to the amount of sugar added. If certain sugar concentrations are exceeded the degree of inhibition becomes smaller.

It is at present not possible to define clearly the mechanism of this inhibition. But sufficient data are at hand, in the literature on glycogenolysis to suggest certain conclusions. Thus the work of Schäffner,[8, 9] Cori,[10, 11, 12] Kiessling[13] and Lehmann[14] demonstrates that the first steps in glycogenolysis are: the phosphorylosis to glucose-l-monophosphate, conversion to the 6-ester and further transformation to fructose diphosphate. Glucose has been shown to inhibit the first step in this chain.[14, 15]

Summary and Conclusions. It is concluded that the inhibition of the rate of appearance of free sugar in glycogenolyzing liver brei, probably depends upon an inhibition of the first step in glycogenolysis, namely, the phosphorylosis of glycogen to glucose-l-monophosphate. These results confirm the previously postulated intrinsic hepatic homeostatic mechanism which contributes to blood sugar regulation by determining the rate of sugar output by the liver. Since it occurs *in vitro,* the non-essential nature of extra insulin secretion for this regulatory activity is confirmed.[3] The actual relationship of insulin and other hormones to this mechanism has already been indicated.

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8 Schäffner, A., and Specht, H., *Naturwiss.*, 1938, **26**, 494.

9 Schäffner, A., *Naturwiss.*, 1938, **27**, 195.

10 Cori, G. T., and Cori, C. F., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 337.

11 Cori, G. T., Colowick, S. P., and Cori, C. F., *J. Biol. Chem.*, 1939, **127**, 771.

12 Cori, C. F., Schmidt, G., and Cori, G. T., *Science*, 1939, **89**, 464.

13 Kiessling, M., *Naturwiss.*, 1939, **27**, 129.

14 Gill, P. M., and Lehmann, H., *Biochem. J.*, 1939, **33**, 1151.

15 Lehmann, H., *Nature*, 1938, **141**, 470.

11018 P

Effect of Insulin on Rate of Appearance of Free Sugar in Liver Brei.

M. Taubenhaus, R. Levine and S. Soskin.

From the Department of Metabolism and Endocrinology, Michael Reese Hospital, and the Department of Physiology, University of Chicago.

Jensen[1] and Soskin[2] have recently summarized the literature regarding the action of insulin on the liver. The indirect evidence available favors the view that insulin inhibits glycogenolysis. More direct evidence has been reported by Issekutz and Szende,[3] who demonstrated that perfused livers removed from frogs which had previously received insulin, produced less sugar than did the livers of untreated frogs. Similar, but less well controlled results, were obtained by Siegel,[4] Molitor and Pollak,[5] and Popper[6] by different methods. On the other hand, Lundsgaard, *et al.*,[7, 8] were unable to show that insulin had any action on glycogen breakdown or deposition in the perfused livers of cats and dogs.

In a preceding communication we have shown a quantitative inhibition exerted by added glucose on the rate of appearance of free sugar in glycogenolyzing liver brei. This offered the opportunity for the testing of insulin action on the liver.

Normal, fed dogs were anesthetized with nembutal and a lobe of liver was removed after tying a broad linen band around its base. There was no bleeding and no detectable symptoms of shock. Insulin (1 unit per kg body weight) was then administered subcutaneously or intravenously as desired. Thirty to 45 minutes after the insulin administration, the remainder of the liver was removed. Total carbohydrate and free sugar were determined at varying intervals under constant conditions, as detailed in the preceding paper.[9]

Results and Discussion. The possible influence of the operative

1 Jensen, H., *Insulin*, Commonwealth Fund, 1938, New York.

2 Soskin, S., *Physiol. Rev.*, Jan., 1940, in press.

3 Issekutz, B., and Szende, J., *Biochem. Z.*, 1934, **272**, 412.

4 Siegel, R., *Klin. Wchnschr.*, 1929, **8**, 1069.

5 Molitor, H., and Pollak, L., *Arch. f. exp. Path. u. Pharmakol.*, 1930, **154**, 280.

6 Popper, H., and Wozasek, O., *Z. f. d. ges. exp. Med.*, 1931, **77**, 414.

7 Lundsgaard, E., *Johns Hopkins Hosp. Bull.*, 1938, **63**.

8 Lundsgaard, E., Nielsen, N. E., and Ørskov, S. L., *Skand. Arch. Physiol.*, 1936, **73**, 296.

9 Soskin, S., Levine, R., and Taubenhaus, M., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 689.

TABLE I.
Inhibition of Glycogenolysis in Liver Brei by Dextrose, and by Dextrose + Insulin.

	Time, min	Total* Cho.	Amt* of dextrose added	Appearance of free sugar*		% inhibition	
				without insulin	with insulin	with dextrose alone	with dextrose + insulin
I.	0	2778	0	141	—	—	—
	60	—	0	299	—	—	—
	60	—	94	319	165	0	45
	60	2897	186	267	145	11	52
II.	0	3313	0	254	—	—	—
	60	—	0	1997	—	—	—
	60	—	100	2027	1180	0	42
	60	—	208	1565	1073	22	46
	60	—	418	1229	805	39	60
	60	3410	836	1260	662	37	67
III.	0	4184	0	105	217	—	—
	60	—	0	1317	1269	—	—
	60	—	100	1196	1066	9	19
	60	—	200	1080	892	18	32
	60	—	450	1019	690	23	48
	60	4000	900	446	222	66	83

* All values are mg per 100 g of liver, calculated as for glucose.

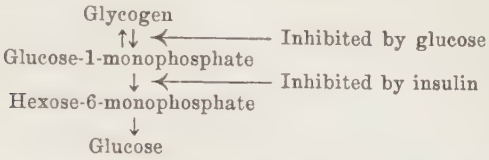
procedure on our results was ascertained by running control experiments in which no insulin was given. There was no difference between the rates of glycogenolysis in the first and second liver samples.

The data summarized in Table I show that in the liver samples removed after insulin administration, there was a significantly lower rate of appearance of free sugar than in the samples removed before insulin was given. When glucose was added *in vitro* to both sets of liver samples, the rate of glycogenolysis was inhibited to a greater extent and by smaller amounts of added glucose in the "insulinized" samples than in the controls.

It was also attempted to reproduce the above effect by adding the insulin *in vitro,* but without success.

The recent work of Gill and Lehmann[10] when related to what is known of the course of glycogen breakdown in the liver, suggests a tentative explanation of the mechanism of the above inhibition. They have shown that insulin inhibits the *in vitro* transformation of the glucose—1-ester to the 6-ester in muscle extract. If this action can be demonstrated to occur in liver, the inhibiting influence of both glucose and insulin could be indicated as follows:

[10] Gill, P. M., and Lehmann, H., *Biochem. J.*, 1939, **33**, 1151.



Summary. Insulin inhibits glycogenolysis in the liver, and reinforces the inhibitory effect of added dextrose.

11019

Influence of Vitamin A upon Urea Clearance in the Rat.*

R. C. HERRIN. (Introduced by W. J. Meek.)

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The study of the effect of vitamin A upon renal excretion was extended to the rat because the diet could be made of simpler materials and avitaminosis A of a more severe degree could be studied economically.

Procedure. A high protein diet was used in order to make the physiological strain on the kidneys greater. It was composed of casein 56, lard 20, starch 16, yeast 5 and Wesson's salt mixture 3. For the first 100 days of the experiment the casein was extracted with ether but as this left appreciable amounts of vitamin A the casein thereafter was dry heated for 2 weeks in the autoclave by passing steam at 15 pounds pressure through the outer jacket of the autoclave. This prolonged heating has been used for many years to free casein of vitamin A. The rats were exposed to light from a quartz Hg vapor lamp to furnish vitamin D. The rats weighed 30 to 40 g when placed on the experimental diet.

The technic of Farr and Smadel[1] with modifications was used in making the urea clearance determinations. The rats were handled for training before being used for clearance study. They were placed in the urine collection cages without a preliminary withdrawal of food. After a urine collection period of 350 to over 600 minutes, blood was drawn from the tail, then the urine was collected for another period of approximately the same length. The same blood

* This study received financial support by grants from the Committee on Scientific Research of the American Medical Association and the Wisconsin Alumni Research Foundation.

1 Farr, Lee E., and Smadel, Joseph E., *Am. J. Physiol.*, 1936, **116**, 349.

TABLE I.
Urea Clearances in Rats in Avitaminosis A.

Rat No.	Date	Urea excreted, per min, mg	Blood urea mg per 100 cc	Surface area in M^2	Urea clearance per M^2 of body surface
2	6-24-37	.293	61.4	.0284	16.8
		.241			13.8
	7-14	.242	39.5		21.6
		.245			21.8
	7-22	.179	67.0		9.5
	7-24 dead				
9	6-1	.265	51.7	.0254	20.2
		.318			24.2
	7-14	.296	68.8		17.0
		.218			12.5
	7-21	.180	213.5		3.4
		.162	114.6		5.6
	7-23	.135	114.6		4.6
	7-27 "				
6	5-26	.139	33.4	.0215	19.4
	6-1	.208	103.3		9.3
	6-6	.186	88.7		9.7
	6-25 "				
1	6-24	.218	92.9	.0267	8.8
		.174			6.7
4	7-14	.160	80.2	.0230	10.5
		.192			8.7
	7-21 "				
12	5-26	.260	34.2	.0262	29.1
	6-27	.438	29.5		55.4
	6-28	.080	29.5		18.5
	6-28 "				
13	5-19	.025	33.3	.0248	3.0
	7-21	.253	56.5		19.2
	7-21 "				
14	6-1	.205	49.0	.0257	16.4
		.323			25.7
	6-5	.231	52.1		17.3
		.254			18.9
	7-27	.236	73.8		12.5
	7-28 "				

concentration of urea was used in calculating the clearances for the two periods. In most cases the first period gave a higher clearance than the second. The former represents a post-prandial clearance and the latter, one of a post-absorptive state. Urine flow was maintained by administering 0.2% NaCl by stomach tube at the rate of 1.0 cc per hour during the period of urine collection.

Blood urea was determined by the micro-manometric method,

TABLE II.
Influence of Carotene upon Urea Clearance in Rats.

Rat No.	Diet	Date	Urea excreted, per min, mg	Blood urea mg per 100 cc	Surface area in M^2	Urea clearance per M^2 of body surface
3	Basal	7-23-37	.386	100.4	.0268	14.3
			.250			9.7
	" + carotene	7-24				
		8-10	.461	56.8		30.3
			.333			21.9
5	"	6-24	.226	54.9	.0287	14.4
			.313			19.9
	" + carotene	7-24				
		8-11	.313	60.3		18.1
			.264			15.3
10	"	6-24	.408	66.3	.0249	24.7
			.277			16.8
		6-28	.524	60.2		19.6
		7-23	.237	44.6		21.3
			.143			12.9
	" + carotene	7-26				
		8-10	.429	42.7		35.6
			.336			31.6
11	"	6-1	.212	73.5	.0259	11.1
			.330			17.4
		6-5	.242	149.8		6.2
			.384			9.9
		6-27	.472	75.2		24.3
			.454			23.3
	" + carotene	7-6				
		8-10	.360	56.0		24.8
			.341			23.6
18	"	7-20	.427	75.6	.0296	19.1
			.331			14.8
		7-23	.327	63.9		17.3
			.237			12.5
	" + carotene	7-24				
		8-10	.485	72.9		22.5
			.384			18.3
20	"	7-14	.361	50.0	.0273	26.7
			.334			24.5
		7-21	.240	85.2		10.3
			.188			8.1
		7-23	.209	53.8		14.3
			.145			9.8
	" + carotene	7-28				
		8-11	.347	55.3		23.0
			.233			15.5
21	"	7-14	.260	52.2	.0253	19.6
			.204			8.3
		7-20	.326	91.5		14.0
			.224			9.7
		7-23	.280	76.4		14.4
			.249			12.5
	" + carotene	7-28				
		8-10	.544	55.1		39.0
			.352			25.3

using urease.[2] The urine urea was determined by the urease, manometric method.[2]

Results. Table I shows the urea clearances on rats as they develop avitaminosis A. The latest clearance was taken a few days before death. All clearances are expressed in cc of whole blood/min per square meter of body surface

In 9 rats the urea clearance, a few days before death or before vitamin A administration began, had decreased 21 to 72% of the normal level. In Rats 1 and 4 a normal clearance was not obtained but the clearances obtained a few days before terminus were about half of the normals in the other rats. Rats 2, 12 (Table I) and 11 (Table II) showed a very great increase in urea clearance when they neared terminus, just as we have observed in young dogs.[3] The clearance of Rat 5 was the only exception, in that it remained within normal limits in avitaminosis A. The urine of rats displaying extreme avitaminosis A showed very little deviation from normal. Occasionally a trace of albumin was found and a few red and white cells. There was nothing to indicate renal damage. Histological sections of the kidneys showed no significant deviation from the normal, which observation confirms Wolbach and Howe.[4] Table II shows the effect of carotene upon urea clearance in avitaminosis A.

Avitaminosis A in 14 of 15 rats resulted in a marked decrease in urea clearance at some time in the course of the deficiency. In those not treated with carotene the percentage of decrease ranged from 23 to 77. Farr and Smadel[1] found that the average urea clearance in their normal rats was 10.9 cc. The average normal of the rats used in this study was 19.3 cc. This greater value is probably due to the high protein content of our diet. Notwithstanding the high protein diet, in 7 rats the clearance was less than 11 cc in avitaminosis A. Rats 11 (Table II) and 12 (Table I) showed a very great increase in urea clearance when they neared terminus, just as we have observed in young dogs.[3]

Administration of carotene equivalent to 100 units of vitamin A daily in 5 of 7 rats (Table II) resulted in a 30 to 170% increase in urea clearance. Rat 11, which did not seem to respond to carotene had had a very great elevation just prior to the addition of carotene. The second exception was Rat 5, whose clearance had not shown a very great fall in avitaminosis A. The range of clearance after

[2] Peters, John P., and Van Slyke, Donald D., *Quantitative Clinical Chemistry*, Vol. II, The Williams and Wilkins Company, pp. 376, 360.

[3] Herrin, R. C., and Nicholes, H. J., *Am. J. Physiol.*, 1939, **125**, 786.

[4] Wolbach, S. Burt, and Howe, Percy R., *J. Exp. Med.*, 1925, **42**, 753.

carotene, varied from 15.5 to 39 with 4 rats having clearances above 21.

Summary. Vitamin A in the diet affects the magnitude of urea clearance in the rat. In avitaminosis A there is a 23 to 77% decrease in urea clearance. Urine examination and histological sections of the kidney showed no marked morphological alteration. It is a functional deficiency.

Administration of carotene in 5 of 7 rats resulted in a 30 to 170% increase in urea clearance above the level in avitaminosis A.

11020

The Potential Produced by Cardiac Muscle. A General and a Particular Solution.

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Let us consider a mass of cardiac muscle immersed in an extensive homogeneous volume conductor. The value of the potential at a point in the conductor, produced by any given distribution of depolarization or repolarization may be obtained theoretically in the following way:

Let v_2 denote the particular region or volume of the muscle mass which is undergoing depolarization at a given instant. Let us choose any point O as the origin of a rectangular coördinate system X, Y, Z. Let $(X_2\ Y_2, Z_2)$ be any convenient point within the region v_2. Let dv_2 be an element of volume of v_2 at the point (X_2, Y_2, Z_2). Let the magnitude of the vector $\mathbf{\emptyset}$ represent the intensity of depolarization of the element dv_2, and let the direction of $\mathbf{\emptyset}$ be that of a line drawn from the effective negative toward the effective positive ionic charge within the element dv_2. The vector quantity $\mathbf{\emptyset}dv_2$ is then the electric moment of depolarization.

Let us choose next any other (fixed) point (X_1, Y_1, Z_1) within the volume conductor, in the vicinity of the muscle mass, at which it is desired to know the value of the potential V due to the distribution of depolarization v_2. Let $\mathbf{r}_1$ and $\mathbf{r}_2$ be radius vectors drawn from O to the points (X_1, Y_1, Z_1) and (X_2, Y_2, Z_2) respectively. Let $\mathbf{r}$ be a vector drawn from the latter to the former point so that $\mathbf{r} = \mathbf{r}_1 - \mathbf{r}_2$. Since the elementary potential dV at (X_1, Y_1, Z_1) due

to the elementary distribution dv_2 varies inversely with the square of the distance r and directly with the cosine of the angle $(\mathbf{r},\boldsymbol{\emptyset})$, we have $dV = \mathbf{r}.\boldsymbol{\emptyset}dv_2/r^3$. Consequently,

$$(1) \ldots\ldots \qquad V = \iiint \frac{\mathbf{r}.\boldsymbol{\emptyset}}{r^3}\, dv_2 = \text{MAX}\boldsymbol{\emptyset}^*$$

where the triple integral is to be taken over the whole of the volume v_2. The validity of this relation is based upon a proper interpretation[2] of the membrane theory of cell excitation; upon the fact that a potential difference or electromotive force assumedly produced by an ionic charge distribution across a cell boundary may be represented by a vector or distribution of vectors; and upon an adequate demonstration[2] that certain well known laws which describe the electric field in volume conductors apply to the circumstances involved with sufficient accuracy to be useful. Equation (1) fails to take into account the finite boundary conditions of the medium surrounding the mass of cardiac muscle. Since the extent of this conductor is great, however, in comparison with all required values of r, the effect of the finite boundary upon the value of the potential is the same or nearly the same as would be the case if the extent of the conductor were infinite.

A similar relation must hold for the value of the potential produced by any given distribution of repolarization. In which case the quantity $\boldsymbol{\emptyset}dv_2$ becomes the electric moment of repolarization, and v_2 becomes the volume distribution of repolarization.

It has been shown theoretically that if the vector $\boldsymbol{\emptyset}$ represents the intensity of depolarization or repolarization during excitation of cardiac muscle, the Maxwellian of $\boldsymbol{\emptyset}$ gives the electric potential at the point (X_1, Y_1, Z_1) due to any distribution of depolarization or repolarization as the case may be. Let us illustrate the application of this relation to a particular electrocardiographic problem.

The accompanying curve represents the successive values of a series of potential differences produced at the tips of 2 nonpolarizable electrodes by the passage of a region of depolarization (accession wave[3]) and region of repolarization (regression wave[3]) along a linear strip of turtle ventricle immersed in a large bath of normal saline. The recording was made with a galvanometer of

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* See reference 1.

1 Vector Analysis, Gibbs, Wilson, E. B., Yale Univ. Press, 7th printing, 1931.

2 Wilson, F. N., Macleod, A. G., and Barker, P. S., Univ. of Mich. Press, Sc. Series 10, 1933.

3 Macleod, A. G., *Am. Heart J.*, 1938, **15**, 165.

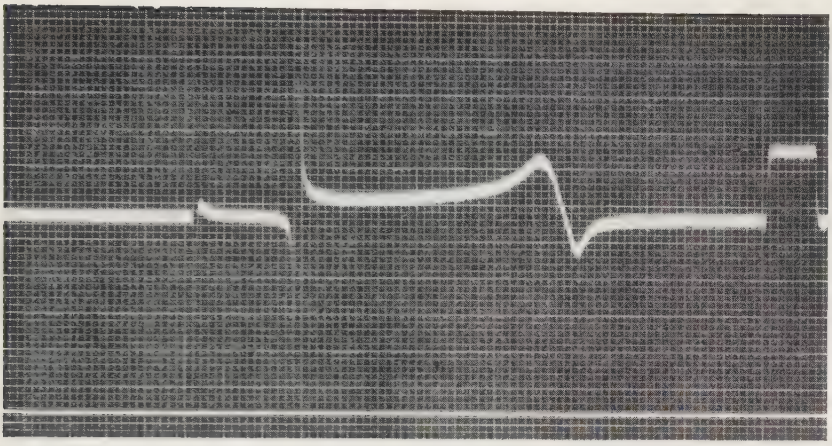

FIG. 1.

the Einthoven type. The contacts of the electrodes were attached to the terminals of the galvanometer in such a way that a positive potential at the tip of the exploring electrode E_1 produced a downward movement on the completed record. Let us denote the left hand end of the muscle strip by A and the right hand end by B. The tip of E_1 was placed near the surface of the muscle strip between AB in the vicinity of B, and corresponds to the point (X_1, Y_1, Z_1). The indifferent electrode E_2 was placed at a remote point in the bath 14 cm distant from the mid-region of the muscle strip. Under these circumstances, the potential V_1 at E_1 due to any distribution of depolarization or repolarization is sufficiently great in comparison with a simultaneous value of V_2 at E_2 that the latter may be neglected.[4, 5]

The left hand deflection, a sharp downward movement, is the artificial effect of stimulation at A with a galvanic current. The right hand upward movement is the usual standardization deflection produced by the introduction of 1 millivolt into the circuit. As the accession wave passed from A to B along AB, beneath the tip of the electrode E_1, the tall rapid diphasic accession deflection was recorded. As the regression wave passed along AB in the same direction a bit later, the low broad diphasic regression deflection was recorded. The order of the polarity of the two diphasic deflections is unlike. Thus the form of the regression deflection is similar to that anticipated by Wilson, *et al.*[2]

4 Wilson, F. N., Wishart, S. W., and Herrmann, G. R., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 276.

5 Wilson, F. N., *Am. Heart J.*, 1930, **5**, 599.

Let us denote by v_o the uniform velocity of the accession wave, and by T the interval of time between the beginning and the completion of depolarization at a given point. The product v_oT then defines the length of the accession wave. It is also one of the 3 dimensions of v_2. Because of the extremely small value of v_oT* in comparison with the minimum value of r, we may look upon v_2 as a surface rather than a volume. Consequently, in the case of accession, dv_2 is equivalent to da_2, where the latter is an element of surface of the accession wave. Hence

(2) . . . . $$\text{MAX}\emptyset = \iint \frac{r.\emptyset}{r^3}\, da_2$$

or

(3) . . . . $$V_1 = \emptyset\Omega$$ Wilson[6]

where Ω is the solid angle subtended at (X_1, Y_1, Z_1) by the boundary of $a_2 (= v_2)$. Inasmuch as depolarization has been considered instantaneous, its rate throughout the distance v_oT is immaterial, and we are thus permitted to regard $\emptyset$ as constant when performing the integration in equation (2). Furthermore, it is obvious from equation (3) that when we ascribe an appropriate value for the constant $\emptyset$, and propagate the distribution a_2 along AB with a uniform velocity v_o, the values of V_1 for successive instants of time will describe a curve similar in all essential particulars to that of the diphasic accession deflection. The fact that the final downstroke of the accession deflection does not return to the base line according to the description of equation (3) is due to the flow at this time of a current artificially introduced into the circuit to neutralize the effect during diastole of an electric field of injury.

Other applications of the expression MAX$\emptyset$ to problems of this general kind are available but their discussion is beyond the limits of this report.

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6 Wilson, F. N., Macleod, A. G., and Barker, P. S., *Am. Heart J.*, 1931, **6**, 637.

* See reference 2.

11021

Failure of Wheat Germ Oil to Produce Neoplasms.

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Rowntree[1] and his associates claimed that intraperitoneal sarcomas developed in rats fed on a crude ether extracted wheat germ oil. The experiment has since been repeated in a number of laboratories with negative results. Those who have reported failure include Carruthers,[2] Day and his associates,[3] Evans and Emerson,[4] and Dingemanse and van Eck.[5] Because Rowntree continues[6] to maintain that sarcomas can be produced by this method, we wish to report our own experience in the matter.

We attempted to repeat as exactly as possible, the original experiment described by Rowntree. The wheat germ oil was prepared according to the method used by E. R. Squibb and Sons in making the oil used by Rowntree and Dorrance for their experiment. Our wheat germ was purchased from Pillsbury Flour Mills Co., in New York. Its extraction was carried out in 2 large Soxhlet extractors. The ether was boiled in a 2-liter round flask and condensed by a large water-cooled bulb condenser into the midsection of the extraction chamber which contained a large paper thimble holding approximately one-third of a pound of wheat germ. The extraction chamber was allowed to siphon over 2 or 3 times, before inserting a fresh supply of wheat germ in the thimble. In this manner 750 pounds of wheat germ were extracted with 267 liters of ether. The ether used was that put up in copper lined cans for use as an anesthetic by E. R. Squibb and Sons. This was the same form of ether

1 Rowntree, L. G., Steinberg, A., Dorrance, G. M., and Ciccone, E. F., *Am. J. Cancer*, 1937, **31**, 359.

2 Carruthers, C., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 107.

3 Day, H. G., Becker, J. E., and McCollum, E. V., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 21.

4 Evans, H. M., and Emerson, G. A., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 318.

5 Dingemanse, E., and van Eck, W. F., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 622.

6 Rowntree, L. G., Steinberg, A., and Brown, W. R., Paper read at the 32nd Annual Meeting of the American Association for Cancer Research, Richmond, Virginia, April 6, 1939.

used in the extraction of the wheat germ oil prepared for Rowntree by E. R. Squibb and Sons.* This form of ether is generally considered to be peroxide-free.

The extract thus obtained was filtered through filter paper to remove the fine particles of wheat germ, and washed with one-half volume of 4% sodium hydroxide solution. It was then washed 4 or 5 times with water. A clear solution resulted. This was distilled at 36° at atmospheric pressure. When most of the ether had been removed the solution was distilled in a vacuum, and a stream of carbon dioxide passed through the oil to remove the last traces of the ether. The temperature was finally increased to 60° to 70° for 1 to 2 hours.

The oil obtained was perfectly clear. It was stored in a refrigerator. After 24 hours of standing in the cold an insoluble precipitate formed. Before the oil was used this material that had separated out was stirred up and dissolved at room temperature, and the whole fed to the rats.

A total of 15 liters of wheat germ oil were extracted in this manner from approximately 337,000 g of wheat germ, during the period from February to May, 1938. The yield, it will be noted, was approximately 4%. The task was a tedious one, but was carried out with all possible care.

Twenty male rats of the Wistar strain, which was the same strain that Rowntree used, were fed the oil in the manner which gave him the best success. Four cubic centimeters of it were poured daily over the portion of food consumed by each rat. The food was a stock diet containing the following ingredients: rolled oats 15 parts; cracked corn 60 parts; dried meat scraps 14 parts; powdered milk 10 parts; sodium chloride 1 part. To this was added 1.25% of cod-liver oil by weight. Water was given freely in drop bottles.

The feeding of the wheat germ oil was begun on July 27, 1938, and was ended when the supply of oil was exhausted on March 28, 1939. Four of the rats died during the course of the experiment, one on February 2, 1939, another on February 28, 1939, and two on March 28, 1939. Autopsy showed pneumonia to be the cause of death in each instance. There were no sarcomas in the peritoneal cavity or elsewhere.

The remaining 16 rats were sacrificed on July 22, 1939. No sarcoma was found in any of them. The only remarkable feature at autopsy was the unusual amount of fat in the omentum, mesentery, and retroperitoneal regions.

* Private communication from Dr. Anderson of E. R. Squibb and Sons.

Summary. A careful repetition of Rowntree's experiment in which ether extracted wheat germ oil was fed to rats failed to confirm his claim that intraperitoneal sarcomas can be induced in this manner.

11022 P

Action of a Quaternary Ammonium Type of Wetting Agent on Metabolism of Microörganisms Associated with Dental Caries.

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In the search for a compound which can effectively inhibit the metabolism of microörganisms found in lesions of dental caries, or in plaques associated with such lesions, we have studied the action of alkyl dimethyl benzyl ammonium chloride (Zephiran*) on these bacteria. The germicidal action of this compound was described by G. Domagh.[1]

Pure cultures of 5 microörganisms, which are found constantly or very frequently in association with human dental caries, were studied. These were a lactobacillus, 2 strains of *M. tetragenus, Staphylococcus albus,* an unidentified, aerobic, acid-producing, Gram-positive diplococcus and a yeast of the genus Monilia. Washed suspensions of the organisms were placed in appropriate buffers containing 0.02M glucose, and their rate of respiration or glycolysis measured in Warburg vessels. The alkyl compound exerts a pronounced inhibitory effect on both respiration and glycolysis at extremely low dilutions (concentrations estimated at M/10,000 to M/50,000).† At a concentration of M/10,000 the compound exerts its full effect on one billion cells within 5 to 10 minutes. That the inhibition is complete and irreversible is demonstrated by the following experiment: organisms which were exposed to the compound for several minutes, and then centrifuged off and washed with

* The authors are indebted to the Alba Pharmaceutical Company for the Zephiran compound employed in this study.

1 Domagh, G., *Deutsche Med. Wochenschr.,* 1935, **61**, 829.

† The molecular weight of the alkyl dimethyl benzyl ammonium chloride compound was approximated as 350 by taking an arithmetic average of the alkyl groups which vary from C_8 to C_{18}. The "compound" is a mixture of the quaternary ammonium derivatives of fatty acids obtained from coconut oil.

TABLE I.

	Micrograms per 10^9 cells	% inhibition (in 60 min)
*Lactobacillus sp.**	25	90
"	4	34
M. tetragenus (A)*	10	97
" (B)‡	2	90
Staphylococcus albus‡	14	94
"	7	94
Gram-pos. oral diplococcus*	15	91
Monilia sp.	3000	89
	600	50

* Aerobic glycolysis.
‡ Respiration.

saline, did not regain their metabolic activity when resuspended in fresh glucose-buffer solution. Table I shows the extremely small quantities of the compound required to inhibit one billion cells of the particular organisms studied.

It can be seen that the alkyl dimethyl benzyl ammonium chloride has an activity comparable to that of the unusually potent compounds recently obtained by Dubos[2] from soil bacteria.

The marked inhibition of the metabolism of dental bacteria by the alkyl dimethyl benzyl ammonium chloride makes this compound of unusual interest. It has been shown by one of us[3] that fluoride and iodoacetate can retard, and in some cases prevent, the development of experimental dental caries in the rat. We have compared the action of "Zephiran" with fluoride and iodoacetate and have found it much more active in inhibiting the metabolism of dental bacteria and yeast. Because of the unusual inhibiting action of the alkyl dimethyl benzyl ammonium chloride compound, its excellent powers of penetration and cleansing, and its low toxicity for mucous membranes, this substance appears to deserve considerable study. The action of "Zephiran" on the mixed bacterial flora of the dental plaque *in vitro* and *in vivo* is also under study.

[2] Dubos, R., *J. Exp. Med.*, 1939, **70**, 1.
[3] Miller, B. F., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 389.

11023

Recovery of Vitamin C from the Human Bladder.*

Sol Sherry and Gerald J. Friedman. (Introduced by Elaine P. Ralli.)

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In the course of observations on the urinary excretion of vitamin C the question arose as to the stability of vitamin C in bladder urine. It has been shown in reports from this[1] and other laboratories[2, 3] that the vitamin C content of drawn bloods remains unchanged for periods up to 24 hours. It is well known, however, that the vitamin C content of voided urine diminishes rapidly unless suitable means of preservation are used. Whether or not the vitamin C content of bladder urine remains unchanged has apparently not been considered.

In order to answer this question, several experiments were done in which vitamin C solutions of known concentration were instilled into the bladder and then later removed at varying intervals, and analyzed. The subjects were patients whose urines contained insignificant amounts of vitamin C and in whom the blood plasma concentration of vitamin C was very low. On the morning of the experiment, the patients were catheterized, the urine discarded and the bladder washed with 20 cc of sterile saline and then 20 cc of air. The catheter was then clamped. After a one-hour control period the clamp was opened, the urine removed, and the bladder again washed with saline and air. As the excretion of vitamin C at low plasma levels is constant,[4] we assumed that the hourly excretion during the remaining period of the experiment would be approximately the same as during the control period. Therefore, the mg of vitamin C excreted during the one-hour control period was multiplied by the number of hours during which the solution of vitamin C was allowed to remain in the bladder, and this amount was subtracted from the total amount recovered. Ten cc of a standard vitamin C solution (prepared immediately prior to use) were instilled into the

* This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation.

1 Friedman, G. J., Rubin, S. H., and Kees, W., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 358.

2 Farmer, C. J., and Abt, A. F., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 399.

3 Cushman, M., and Butler, A. M., Proc. Soc. Exp. Biol. and Med., 1938, **39**, 534.

4 Friedman, G. J., Sherry, S., and Ralli, E. P., in press.

TABLE I.

Exp.	Plasma Vit. C, mg %	Vit. C instilled, mg	Time in bladder, hr	Amt of urine excreted, cc	Vit. C recovered, mg	Vit. C control, mg	Corrected Vit. C recovered, mg	% recovery
I	.12	0.486	1	260	0.459	.000	0.459	94.5
II	.12	0.528	1	178	0.485	.000	0.485	92.0
III	.10	29.9	3	197	32.2	.120	32.1	107
IV	.03	10.9	5	334	10.8	.142	10.7	98.2
V	.00	9.55	5	232	10.7	.460	10.3	107
VI	.03	4.70	3	205	4.70	.078	4.62	98.3

bladder from a pipette through the catheter and the catheter washed through with 20 cc sterile saline, and then clamped. After varying periods of time the catheter was opened, the urine removed and the bladder washed with sterile saline and air. All specimens were immediately acidified with 10% by volume of glacial acetic acid. Vitamin C was determined in the photoelectric colorimeter by the method described by Evelyn.[5]

In experiments I and II, 10 cc of a 5 mg % solution were placed in the bladder on 2 separate occasions and 94.5% and 92% of the 0.5 mg recovered respectively after a period of one hour. When the same amount of vitamin C was added to the same urine but left standing in the dark at room temperature for the same time (one hour) only 65% was recovered. In experiment III, 10 cc of a 300 mg % (30 mg) solution were instilled into the bladder and left there for 3 hours. The entire amount was recovered at the end of the 3 hours. In experiments IV and V, 10 cc of a 100 mg % (10 mg) solution were placed in the bladder. The recovery figures after 5 hours were 98.2 and 107%. The pH of the urines in all 5 experiments fell between 6 and 7.

The possibility arose that the use of saline might act as a protection against the destruction of the vitamin C instilled into the bladder. The fact that large amounts of urine were excreted into the bladder during the experimental period argued against this but as a further check in experiment VI, vitamin C was dissolved in the urine withdrawn at the end of the control period, and this was reinstilled into the bladder. 4.70 mg of ascorbic acid was injected in this manner and 4.62 mg or 98.3% was recovered at the end of 3 hours.

Summary. When amounts of synthetic ascorbic acid varying from 0.5 to 30 mg were instilled into the human bladder, 92 to 107% was present in the urine after periods of 1 to 5 hours. It is, therefore, concluded that no appreciable destruction of ascorbic acid occurs in the human bladder.

5 Evelyn, K. A., Malloy, H. T., and Rosen, C., *J. B. C.*, 1938, **126**, 645.

11024

Response to Type 1 Pneumococcal Vaccine of Persons Belonging to Different Blood-Groups.*

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Antibody-production following oral administration of pneumococcal vaccine, types 1, 2, and 3, to human beings was reported.[1] Witebsky, Neter, and Sobotka[2] have described an immunological relationship between the specific polysaccharide of type 1 pneumococcus and the blood-group A specific substance.† They observed that isoagglutination of group A human red cells by group O serum, as well as lysis of sheep's red cells by anti-A rabbit serum, was inhibited by acetyl-SSS-1 derived from pneumococci grown in broth which contained peptone. In subsequent experiments[3] in which veal broth free of A-substance was used for propagating the pneumococci they found a less pronounced, though definitely demonstrable, interference. They left open the question whether the phenomenon was due to contamination of SSS with A-antigen from the medium or to similar chemical groupings in the two substances. If due to the latter it might be expected, though it need not necessarily follow, that persons belonging to blood-group A would not form antibody to type 1 pneumococcus so well as members of groups O and B. Similarly it might also be expected that fewer positive skin-reactions would occur among A than among O persons after intracutaneous injection of acetyl-SSS-1, but Rogers and Wagner[6] did not find this to be so.

The present study was made because of the general interest in

* This work was made possible by a grant from Lederle Laboratories, Inc., which the author gratefully acknowledges.

1 Ross, Victor, *J. Immunol.*, 1934, **27**, 307.

2 Witebsky, Ernst, Neter, Erwin, and Sobotka, Harry, *J. Exp. Med.*, 1935, **61**, 703.

† An analogous relationship between type 14 pneumococcal polysaccharide and the human blood-group specific substances has been described.[4, 5]

3 Sobotka, Harry, Witebsky, Ernst, Neter, Erwin, and Schwarz, Eleanor S., *J. Inf. Dis.*, 1937, **60**, 257.

4 Finland, Maxwell, and Curnen, Edward C., *Science*, 1938, **87**, n.s., 417.

5 Hoagland, Charles L., Beeson, Paul B., and Goebel, Walther F., *Science*, 1938, **88**, n.s., 261; *J. Biol. Chem.*, 1939, **129**, 455; Beeson, Paul B., and Goebel, Walther F., *J. Exp. Med.*, 1939, **70**, 239.

6 Rogers, Edw. S., and Wagner, Harold C., PROC. SOC. EXP. BIOL. AND MED., 1935-6, **33**, 249.

any relationship that may exist between blood-group and the capacity to form antibody for bacterial antigens, as well as to learn whether the anticipated consequence of the acetyl-SSS-1 and A-substance relationship would be observed. Although there is evidence that the distribution of blood-groups among persons suffering from various infectious diseases is the same as among the healthy population and there is information concerning the susceptibility to diphtherial and scarlatinal toxins and blood-group there is a lack of data on the response of persons of different blood-groups to the stimulus of an antigen.

The survival of a single mouse infected with one fatal dose of pneumococci was considered evidence of the presence of antibody. Of the 63 persons who were the subjects of the experiments referred to above, blood-group data were obtained for 50. In addition, the groups of 8 of 13 persons to whom only type 1 vaccine had been previously administered were determined.[7] Five of the 50 and 1 of the 8 were not included in calculating the percentage of individuals that formed antibody for type 1 pneumococcus, either because the concentration of native antibody for this organism was so high that a response even if it had been maximal, could not have been detected (4 cases), or because type 1 vaccine was not given‡ (1 case) or due to insufficient serum to make the protection-test (1 case). Of the remaining 52 persons 19 belonged to group O, 24 to group A, 7 to B and 2 to AB. Fourteen individuals in group O, 16 in group A, 6 in group B and both AB individuals formed antibody following ingestion of type 1 vaccine. There appears to be no significant difference between persons belonging to the 2 blood-groups (O and A) comprising the large majority of this series of subjects so far as capacity to react to the vaccine is concerned. The total number of B and AB subjects is too small to justify emphasizing the better response. If there are common chemical groupings in SSS-1 and the specific antigen of human A-cells their presence is, apparently, insufficient to interfere with the production of antibody for type 1 pneumococcus.

The data also give some information regarding the frequency of native type 1 pneumococcal antibody among persons belonging to the several blood-groups. Of the total of 58 typed individuals there were 22 O's, 25 A's, 7 B's, and 4 AB's. Eight persons belonging to group O, 4 to A, 4 to B, and 2 to AB possessed such antibody. A

7 ROSS, Victor, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 822.

‡ This subject is recorded because his blood-group is included in the calculation of the percentage of individuals possessing native pneumococcal antibody.

much larger number of persons will have to be examined before the difference between groups O and A can be accepted as other than fortuitous.

Summary. Persons belonging to blood-group A and those belonging to blood-group O formed protective antibody equally well following ingestion of type 1 pneumococcal vaccine. A somewhat greater percentage of persons in group B responded than in groups O and A but the number of individuals was too small to justify relating the fact to membership in the group.

The author wishes to express his thanks to Dr. Ralph S. Muckenfuss, Director of the Bureau of Laboratories of the Department of Health, for providing the facilities which made it possible to carry out these experiments.

11025

Effect of Desoxycorticosterone Acetate upon Plasma Volume in Patients During Ether Anesthesia and Surgical Operation.

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In recent years, several workers have found that hemoconcentration develops in the course of ether anesthesia. McAllister[1] showed that in dogs there is a drop in plasma volume and a rise in hematocrit. Searles[2] employing an increase in hematocrit, red cell count, and hemoglobin as his criterion for hemoconcentration in dogs came to a similar conclusion. Bollman, *et al.*,[3] also working with dogs, were able to demonstrate a fall in plasma volume and rise in hematocrit in response to ether anesthesia. Gibson and Branch[4] showed that ether anesthesia in man was accompanied by a slight but definite decrease in plasma volume. Stewart and Rourke[5] showed in man

1 McAllister, F., *Am. J. Physiol.*, 1938, **124**, 391.

2 Searles, P. W., *Am. J. Surg.*, 1938, **41**, 399.

3 Bollman, J. L., Svirbely, J. L., and Mann, F. C., *Surgery*, 1938, **4**, 881.

4 Gibson, J. G., 2nd, and Branch, C. D., *Surg. Gyn. and Obst.*, 1937, **65**, 741.

5 Stewart, J. D., and Rourke, G. M., *J. Clin. Invest.*, 1938, **17**, 413.

that surgical procedures are in some instances associated with a fall in plasma volume as determined post-operatively.

Barbour and Bourne[6] felt that the degree of hemoconcentration in dogs brought about by ether anesthesia could be diminished by the preanesthetic injection of fluid. McAllister and Thorn[7] were able to show that the hemoconcentration induced by ether anesthesia in dogs (as evidenced by rise in hematocrit and fall in plasma volume) was overcome by the preanesthetic injection of adrenal cortical hormone. The present work was undertaken in order to determine whether or not adrenal cortical hormone might have a similar effect upon possible changes in plasma volume during ether anesthesia and operation in man.

Plasma volume was determined by the method of Gregersen, Gibson, and Stead.[8-12] Reinjection of the dye at the end of the experiments was not done in this study, as it has been shown (Hamlin and Gregersen,[13] McAllister[3]) that changes in the disappearance curve mirror changes in plasma volume. Hematocrit was determined by using one cc hematocrit tubes spun at a standard rate for a constant length of time employing heparinized blood. Serum protein was measured by means of a Zeiss refractometer. Changes in serum protein were checked in a few instances by the micro-kjeldahl method. Plasma volume was measured one to three hours before operation.*

The patients studied were from the Squier Urological Service. Although an attempt was made to select patients undergoing only minor surgical procedures, this was not at all times feasible, and some of the results are complicated by such factors as blood loss and traction on the kidney pedicle. Anesthesia was administered by the regular anesthetists of the hospital. Preoperative medication consisted of morphine and atropine. In no instance did this medication alone cause a change of plasma volume. Anesthesia was induced in all the patients with nitrous oxide and oxygen and this was followed by the administration of ether. When anesthesia was con-

6 Barbour, H. G., and Bourne, W., *Am. J. Physiol.*, 1923, **67**, 399.

7 McAllister, F., and Thorn, G., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 736.

8 Gregersen, M. I., Gibson, J. G., 2nd, and Stead, E. A., *Am. J. Physiol.*, 1935, **113**, 54.

9 Gregersen, M. I., and Stewart, J. D., *Am. J. Physiol.*, 1939, **125**, 142.

10 Gregersen, M. I., and Schiro, H., *Am. J. Physiol.*, 1938, **121**, 284.

11 Gregersen, M. I., and Gibson, J. G., 2nd, *Am. J. Physiol.*, 1937, **120**, 494.

12 Gregersen, M. I., *J. Lab. and Clin. Med.*, 1938, **23**, 1.

13 Hamlin, E., and Gregersen, M. I., *Am. J. Physiol.*, 1939, **125**, 713.

* We wish to thank Dr. M. I. Gregersen for furnishing us with a supply of the blue dye, "T 1824."

sidered deep enough to permit incision, a blood sample was taken without stasis. Another sample of blood was taken during the operation and a third when the patient returned to the ward. Varying amounts of desoxycorticosterone acetate† were administered subcutaneously for 45 minutes to 8 hours preoperatively, or 20 to 30 cc of cortin† were injected immediately before anesthesia.

Results. The results are shown in Fig. 1. Seven patients served as controls. All showed a fall in plasma volume with ether anesthesia alone. The magnitude of this fall as measured by the dye, serum protein, and hematocrit is slight and in all but 2 patients is within the limit of error of the method. However, during the period of operation itself, 5 of the 7 patients showed a fall in plasma volume exceeding the limits of error. In the postoperative period, one of 7 patients showed a lowered plasma volume. In one patient, the drop in

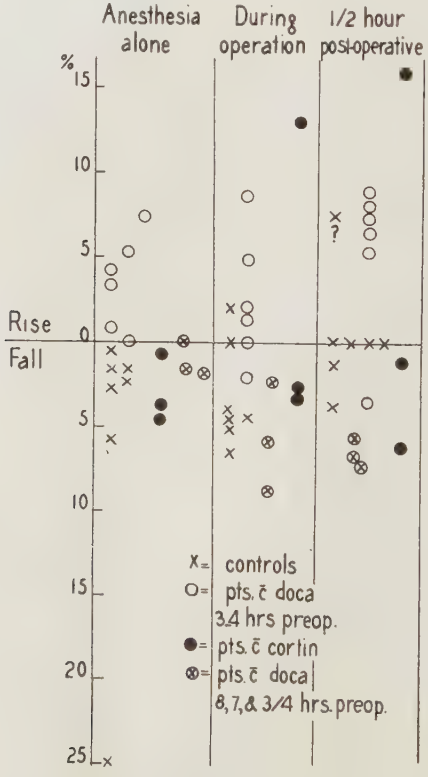

FIG. 1.
Per cent change in plasma volume in operative patients. "Doca" is the term used for desoxycorticosterone acetate.

† Material furnished by Roche-Organon, Inc., Nutley, N. J.

plasma volume as measured by the dye was not confirmed by a drop in plasma volume as measured by the serum protein and hematocrit. In the other 5 patients, there was no change from their preoperative plasma volume.

Nine patients were given between 16 and 20 mg of desoxycorticosterone acetate subcutaneously before operation. In 3 patients in whom the synthetic hormone was given at 8, 7, and ¾ hours respectively preoperatively, there was a fall in plasma volume with ether anesthesia alone of the same order of magnitude as seen in the control group. During the period of the operation, 2 of the 3 showed a significant fall in plasma volume, while in the third the fall was within the error of the method. One-half hour postoperatively, one of the 3 showed a significant fall in plasma volume. In the other 2 patients, the fall in plasma volume as measured by the dye was not supported by the serum protein and hematocrit determinations. In other words, the hormone produced no demonstrable effect in these patients.

In the other 6 patients, the synthetic hormone was given between 3 and 4 hours preoperatively. Three of these 6 showed a measurable increase in plasma volume during the period of ether anesthesia alone. In the other 3, the increase as measured by the dye was either within the limit of error of the method or was not corroborated by a significant increase as measured by the serum protein or hematocrit. During the operative period, one showed a significant increase in plasma volume. In 5, no change was found. Five of the 6 patients showed a significant rise in plasma volume during the postoperative period, while the sixth showed no change. Thus it is apparent in this group that the usual fall in plasma volume was prevented by synthetic hormone given 3 to 4 hours before operation.

Three patients received 20 to 30 cc of cortin intravenously immediately prior to anesthesia. During anesthesia alone, there was the usual insignificant decrease in plasma volume. During the operative period, 2 of the 3 showed a similar insignificant drop and one showed an increase in plasma volume. In the postoperative period, one showed an increased plasma volume, in one there was a minor decrease, and in the third there was a significant fall. In this group, the results are obviously inconclusive.

Summary and Conclusions. (1) Plasma volume was determined in patients undergoing ether anesthesia and surgical operation. Seven patients served as controls, 9 received desoxycorticosterone acetate (subcutaneously) at varying time intervals before operation, and 3 received crude adrenal cortical hormone (intravenously) immediately before the anesthetic. (2) Of the 7 patients serving as con-

trols, 2 showed a significant fall in plasma volume during ether anesthesia alone, 5 during the period of operation, and one, one-half hour postoperatively. In none of these controls was there a significant increase in plasma volume during any of the 3 periods studied. (3) Six patients were given desoxycorticosterone acetate subcutaneously 3 to 4 hours before anesthesia. In this group, 3 patients showed a significant increase in plasma volume during the period of anesthesia alone, one patient during the operation, and 5 patients one-half hour after the operation. There were no patients in this group who showed a significant fall in plasma volume during any of the 3 periods. (4) In this small series of patients who have undergone surgical procedures accompanied by slight blood loss, the decrease in plasma volume associated with ether anesthesia and these surgical procedures is small. This small decrease in plasma volume is not present when patients have been given desoxycorticosterone acetate subcutaneously 3 to 4 hours before operation.

11026 P

Action of Diethyl Ether on Histamine Release in Anaphylaxis.*

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Anesthesia is known to lessen the intensity of anaphylactic shock; sensitized guinea pigs, under anesthesia, are more likely to survive the injection of the antigen.[1, 2] From experiments in which sensitized guinea pig uteri were suspended in urethane solution and did not contract upon addition of the antigen, Farmer[3] recently concluded that: (a) the anesthetic does not prevent the union between antigen and antibody, since the uteri did not contract either upon readministration of the antigen, when the narcotic had been washed out and the response to histamine was restored, and (b) that the anesthetic inhibits the action of histamine released in shock, while it does not interfere with its release. The following is a report on experi-

* Aided by a grant from the John and Mary R. Markle Foundation.

1 Besredka, A., *Ann. de l'Inst. Pasteur*, 1907, **21**, 957.

2 Farmer, L., *J. Immunol.*, 1937, **32**, 195.

3 Farmer, L., *Ibid.*, 1937, **33**, 9.

ments in which the action of diethyl ether on the histamine release in anaphylaxis of guinea pig tissues was studied.

One or two isolated lung lobes, one uterine horn, or one seminal vesicle from guinea pigs previously sensitized to egg-albumen, were incubated according to Schild[4] in small amounts of antigen solution at 37° C for 10 minutes. After boiling in a water bath, the incubation fluid was then assayed for histamine on the atropinized guinea pig's ileum. While these tissues released considerable amounts of histamine when in contact with the antigen proving that the animal was well sensitized and that its tissues were capable of histamine release, the other lung lobes and the second uterine horn or seminal vesicle failed to do so, when they had been previously etherized for periods of 20 to 40 minutes. Incubation in 0.25 to 3% ether in Locke's solution usually resulted in no release, or a greatly reduced one, of detectable amounts of histamine upon subsequent incubation with antigen.

When sensitized guinea pig lungs were perfused from the pulmonary artery with Locke's solution containing from 0.25 to 3% ether, in most instances injection of the antigen into the arterial cannula failed to release histamine in detectable amounts into the perfusate, as unetherized lungs do.[5,6] In these experiments, one "control" lung lobe was removed previous to the perfusion with ether solution and incubated in antigen solution. Its "shock fluid" contained histamine; so did the "shock fluid" from a uterine horn or seminal vesicle from the same animal, proving that the latter was sensitized.

When in a series of experiments the lungs were inflated and deflated by artificial respiration and gaseous ether was introduced via respiratory air, here again in most cases introduction of antigen caused no manifest histamine release. The failure to obtain such results in some instances is held to be due to mechanical factors; in these experiments, one or several of the lobes failed to be ventilated by the artificial respiration, so that certain parts of the lung tissue were probably etherized only incompletely, or not at all.

Further experiments proved that this action of ether was one upon the tissue and not upon the antigenic properties of egg-albumen, which were found to remain intact when antigen solutions, containing ether, were left standing for several hours. A subsequent publica-

4 Schild, H. O., *J. Physiol.*, 1939, **95**, 393.

5 Bartosch, R., Feldberg, W., and Nagel, E., *Pflüger's Arch.*, 1932, **230**, 129, 674.

6 Daly, I. de Burg, Peat, S., and Schild, H., *Quart. J. Exp. Physiol.*, 1935, **25**, 33.

tion will deal with the details of these studies and with a report on the action of ethyl urethane on the histamine release in anaphylaxis of guinea pig tissues.

Although earlier investigations by one of us[7] on arterial muscle, and more recent ones by Farmer,[3] on the uterus, show that some narcotics inhibit the response of smooth muscle to histamine, the results shown above seem to indicate that ether prevents the fatal anaphylactic shock in guinea pigs at least partly by suppressing the release of histamine. Such views are in contrast to Farmer's,[3] who held that anesthesia acts in the secondary stages of anaphylaxis by inhibiting the action of histamine, while it does not interfere with its release.

11027 P

Effect of Varying the Volume of Injection in Calculating Number of Infectious Particles of Vaccinia.

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In the course of certain experiments with vaccinia virus it was found that the injection of a small volume of a virus dilution gave approximately the same number of positive lesions as obtained with a much larger volume. In other words it was not the number of virus particles injected but their concentration which was important. This point was thought of sufficient interest to warrant further study. This paper is a preliminary report on the subject.

Methods and Materials. The purified vaccinia virus used in these experiments was similar to that used in previous experiments.[1] Measured amounts of this virus were rapidly frozen and dehydrated in a Flosdorf-Mudd apparatus and kept sealed in a vacuum until ready for use. The number of virus particles was calculated according to the method described by Parker.[2] For example, from Experiment 1, the log of the dilution which contains 1 particle per 0.1 cc is 6.93; therefore the number of particles in the virus suspension calculated from these data would be 10 times the antilog of 6.93 which

7 Katz, G., *Arch. f. exp. Path. u. Pharm.*, 1929, **141**, 366.

1 Sprunt, D. H., and McDearman, S., in preparation.

2 Parker, R., *J. Exp. Med.*, 1938, **67**, 725.

is 8.5×10^7 infectious units per cc of the virus suspension. All the conditions of the experiment, except the volume of the injections, were kept the same as Parker's.[2] The X^2 for goodness of fit to the 1 particle curve were calculated by using both positive and negative variations as pointed out by Haldane.[3] Adult male rabbits were used for the titration of the virus. Care was taken to put the same number of injections of each size in each rabbit so as to avoid the variation in susceptibility in the rabbits. The difference in susceptibility of skin on various portions of the rabbits was also taken into consideration in a similar manner.

Results. The results are shown in Table I. It will be seen that the injection of amounts larger than 0.25 cc changes the calculated number of virus particles considerably. Amounts smaller than 0.25 cc in some instances increase the number of particles fourfold and in others the increase is only twofold.

These experiments show that the presence or absence of infection with vaccinia virus injected intradermally is not dependent entirely on the number of virus particles injected. The resulting lesions are more closely related to the concentration of the virus particles in the volume injected than to the number.

One possible explanation of this phenomenon is that in the in-

TABLE I.
Table Showing Effect of Using Different Volumes of Injection in Calculating Number of Infectious Particles.

Vol of virus injection	No. of injections of each dilution	Log of dilution containing 1 infectious unit	X^2 for fit to 1 particle curve	P	Calculated No. of infectious units per cc
		Exp. 1.			
0.50	24	6.99	3.14	.80	1.9×10^7
0.10	24	6.93	11.08	.10	8.5×10^7
		Exp. 2.			
0.50	16	7.74	6.79	.40	11.0×10^7
0.10	16	7.68	1.18	.97	47.9×10^7
0.05	16	7.60	2.75	.85	79.6×10^7
		Exp. 3.			
1.00	20	7.98	7.43	.25	9.5×10^7
0.25	24	7.83	2.31	.80	27.0×10^7
0.05	24	7.55	5.37	.50	70.9×10^7
		Exp. 4.			
2.00	18	8.27	5.38	.50	9.3×10^7
0.25	24	8.27	2.28	.90	74.4×10^7
0.05	24	7.78	6.81	.40	120.5×10^7
		Exp. 5.			
1.00	24	8.34	14.42	.75	21.9×10^7
0.25	24	8.23	4.47	.60	67.9×10^7
0.05	24	7.77	3.54	.75	117.8×10^7

[3] Haldane, J. B. S., *J. Hygiene*, 1939, **39**, 289.

jecting of a given volume only a small portion actually stays in the epidermis, the remainder going into surrounding tissues which are not susceptible to this virus. Hence, if more virus is injected than can stay in the skin it has no effect. Up to a certain point as shown in Table I the increase in the volume of injected solution does increase slightly the chance that a susceptible cell will become infected. It should be emphasized that this does not negate the theory that 1 particle causes infection. It does, however, show that an erroneous figure may be obtained in calculating the number of particles in a virus suspension if the volume of injection used is too large.

Although the above explanation at present seems the most likely, further experimentation must be done as there is the possibility that this phenomenon represents a much more fundamental character of virus infection. We are continuing the study of this subject.

Summary. Experiments are reported in which it is shown that under usual experimental conditions the concentration and not the volume of a vaccinia virus dilution is important in determining the number of infectious units contained in a given virus suspension.

11028

Experimental Reversal of Sex in Salamanders by the Injection of Estrone.*

RICHARD J. ACKART AND STANLEY LEAVY. (Introduced by R. K. Burns, Jr.)

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Experimental evidence derived from the work of Burns, Humphrey, Witschi and others[1] has established the fact that differentiation of sex in salamanders may be modified or even completely reversed through the action of sex-differentiating principles produced in the gonads of a parabiotic partner of opposite sex, or by a gonad of different sex type resident as a graft. This report presents briefly the results of experimental sex reversal in the salamander *Amblystoma tigrinum* (Green), during the period of sex differentiation,

* The crystalline substance used was very kindly supplied by the Schering Corporation.

[1] Allen, E. (Editor), 1939, *Sex and Internal Secretions,* 2nd Ed., Chapters III and IV. Baltimore, Williams & Wilkins Co.

induced by treatment with a female sex hormone of *mammalian origin*—crystalline estrone. It seemed of interest to determine whether such hormones are specific in their actions throughout vertebrates in general, and if so whether their effects are exerted through the differentiating mechanisms already demonstrated for amphibians. The latter point might be determined by close comparison of the histological process in the two cases.

Thirty-six larvae of *A. tigrinum* were selected, closely approximating 30 mm in length, at which stage sex differentiation is beginning in this species. Fourteen were reserved as controls, subject to the same environmental conditions as the experimental group. The 22 experimental subjects each received 2 injections weekly of .01 cc of a solution of estrone in sesame oil over a period of 6 weeks. Each injection contained 25 γ of the crystalline substance, the total treatment representing approximately 350 γ. Injections were made into the body cavity by means of a modified microinjection apparatus.

During the experiment individuals were reared in isolation, at a dietary level permitting a relatively slow rate of development, thereby prolonging the period of sex differentiation. At the end of treatment the subjects were still larvae and approximately 65 mm in length.

During the experiment 7 animals died as a result of injury or from unknown causes. Most of these deaths came early in the experiment while the subjects were quite small. From the data on experimental sex reversal in salamanders generally, it can be safely concluded that this mortality was incidental and without significance for the problem at hand. A few specimens subsequently showed a mild form of ascites, due probably to infection admitted to the body cavity while injecting. Some deaths may have been due to this cause.

After preservation, examination by gross dissection at once showed entire absence of typical males in the experimental group. Controls, on the other hand, proved to be distributed equally as to sex—7 males and 7 females. At this stage of development, ovaries are large, irregular and convoluted, or folded along their free borders. Numerous large, growing ovocytes in their follicles give the surface a coarsely granular character. Testes, on the contrary, are much smaller, smooth and spindle-shaped, frequently lightly sprinkled with fine pigment. The two normal types can scarcely be confused at this stage of development. Of the 15 injected specimens, 9 resembled control females so closely, both in form and size of the gonads, as to lead to the conclusion that these individuals were doubtless genetically female, and that experimental treatment had not significantly modified the normal course of ovarian development. The remaining 6

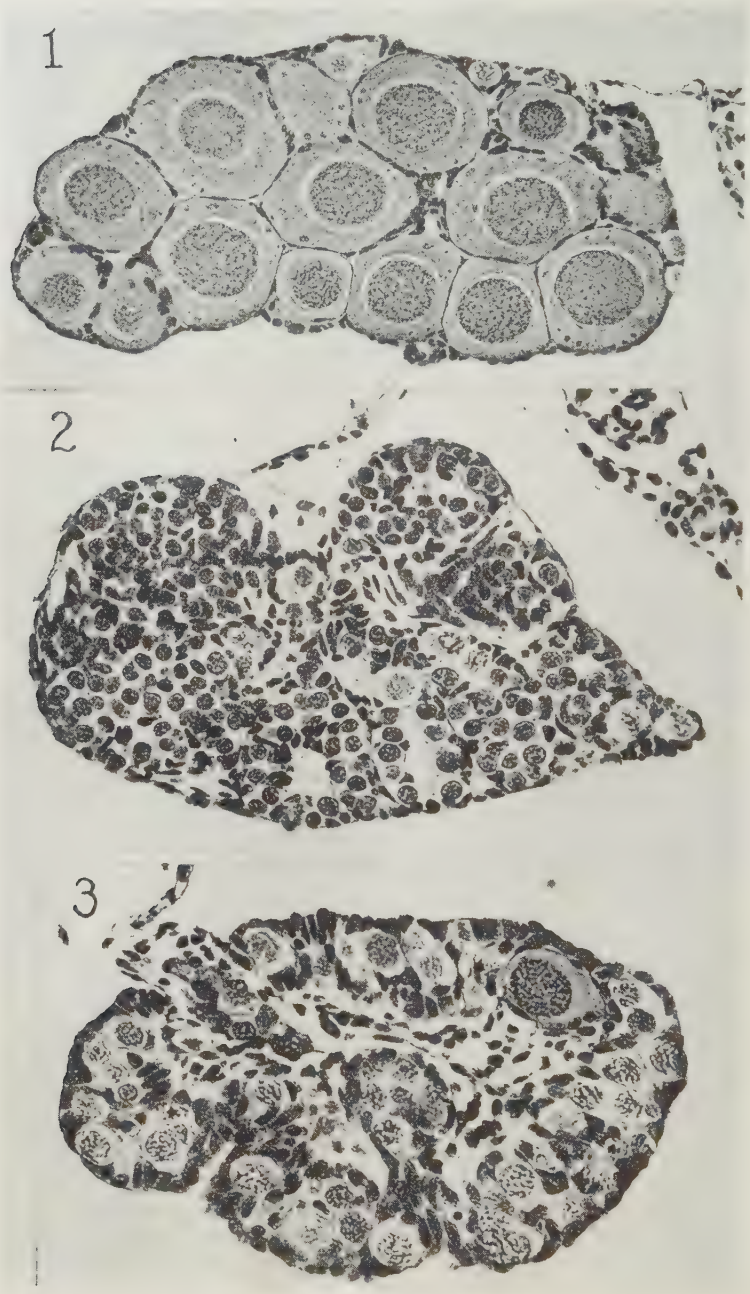

FIG. 1.
Normal ovary of a control specimen, × 150. Large growing ovocytes in their follicular envelopes virtually obliterate the ovarial cavity.

FIG. 2.

Normal testis of a control male, × 150. The germinal elements are in spermatogonial and spermatocyte stages. The medullary substance is compact, with no internal sac.

FIG. 3.

Typical section of an experimentally produced ovo-testis, × 150. The cortical elements have the cytological characteristics of ovocytes. The medullary area is degenerate and shows evidence of cavity formation in the region corresponding to the ovarial sac.

cases varied greatly in size, but none approached the proportions of normal ovaries. All were irregular in form, more closely resembling immature ovaries than testes at any stage of development. Histological study was necessary to determine their real character.

Microscopic examination of these atypical gonads revealed ovo-testes in every case. The rudimentary cortex of the larval testis, under the influence of the hormone, had developed to a condition resembling that of retarded ovaries. Varying amounts of medullary tissue remained, however (Fig. 3). In the cortex of the ovo-testes, the germinal elements had the cytological characteristics of ovocytes, while in the medulla regressive changes, such as degeneration and vacuolization, were usually apparent. In some cases the medullary region had been practically obliterated and the gonads were rudimentary ovaries. In others it remained a definite structure, but cortical development always greatly exceeded that normal for developing testes. The principal difference between the ovo-testes and young normal ovaries consisted in the large amount of medullary stroma in the former, and the more advanced condition of the cortex in the latter. (Compare Figs. 1, 2 and 3.) The type of reversal here described is very similar to that reported by Burns[2] in *A. punctatum,* following estrone injections.

No definite modification of the gonoducts was noted in these animals, due doubtless to the generally immature condition of the subject at the conclusion of treatment, since in somewhat older *A. punctatum* (at metamorphosis) Burns[2] had found a moderate but unmistakable stimulation of the oviducts following estrone administration.

The histological changes produced by injection of estrone are quite similar in detail to the results of parabiotic twinning, and the reversals obtained by Humphrey through gonad grafting. However, Padoa,[3] by adding "folliculin" to the water in which the tadpoles *(Rana esculenta)* were developing, obtained a "paradoxical effect"; namely, the differentiation of males only. Witschi and Crown,[4] using the

2 Burns, Robert K., Jr., *Anat. Record,* 1938, **71**, 447.

3 Padoa, Emanuele, *Arch. Ital. Anat. et Embriol.,* 1938, **40**, 122.

4 Witschi, E., and Crown, E. N., *Anat. Rec.,* 1937, **70**, 121.

same method of administration, failed to observe this paradoxical effect in *Rana pipiens* larvae, but obtained female or hermaphroditic differentiation using the female hormone, dihydrotheelin, and male differentiation after adding testosterone propionate. Except for the above-mentioned result of Padoa, it appears that mammalian sex hormones have a sex-specific effect on amphibian differentiation, at least in the period of larval development. Recently Foote and Witschi,[5] using sexually differentiated larvae of *Rana clamitans,* have caused a secondary transformation of ovaries into testes by injecting testosterone propionate, though they found estrogenic hormones to have little effect on the structure of the testes of these animals. Puckett,[6] however, obtained a precocious sexual differentiation in *Rana catesbiana* tadpoles after injecting pituitary extracts in addition to sex hormones, a result not obtained by injection of sex hormones alone. In his experiment, theelin plus pituitary preparation produced a 100% differentiation of ovaries, while testosterone propionate plus pituitary preparation produced a differentiation of testes in 100% of the animals receiving this combination.

Summary. 1. Twenty-two larvae of *A. tigrinum,* at the beginning of sexual differentiation, were each injected with 350 γ of estrone, distributed over a period of 7 weeks. Seven cases died. 2. Macroscopically, the gonads of 9 treated animals closely resembled those of control females, while the gonads of 6 were atypical. The latter were of smaller size, smoother in contour, and in other respects suggestive of abnormal testes rather than ovaries. 3. Histological examination showed these atypical gonads to be ovo-testes so modified by estrone as to resemble retarded ovaries with irregular medullary remains.

5 Foote, C. L., and Witschi, Emil, *Anat. Rec.*, 1939, **75**, 75.

6 Puckett, W. O., *Anat. Rec.*, 1938, **75** (Suppl.), 89.

11029 P

Effect of Fluorine on Solubility of Enamel and Dentin.

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The effectiveness of fluorine in preventing dental caries has been demonstrated both in man[1] and animals.[2, 3] The finding of a higher fluorine content in caries-resistant teeth[4] indicates that this effect is the result of the incorporation of the fluorine into the teeth. Support for this is found in our unpublished work which shows a reduced acid solubility of fluorine-containing enamel and dentin from incisors of rats fed a high fluorine diet.

The fact that fluorine combines actively with bone,[5] calcium phosphate,[6] and hydroxyapatite[7] suggests that the fluorine may react similarly with the mineral phase of fully formed erupted teeth and give a reduction of solubility like that produced in the calcium phosphates.[6] The following experiments were designed to test this possibility.

Human enamel and dentin powdered to pass a 100 mesh screen were separated and purified by the centrifugal-flotation method.[8] Five hundred milligram samples of enamel were shaken for periods of one hour in 250 cc of solutions containing one part of sodium fluoride in 25, 100, 1000 and 10,000 parts of water, and then thoroughly washed in distilled water. Samples of dentin were similarly treated with 1/1000 sodium fluoride solutions. Solubilities of these and untreated control samples were compared by measuring the respective weight losses of 50 mg samples after one hour in 20 cc of a 0.2 M acetic acid/sodium acetate buffer at pH 4.0.[9]

The results, summarized in Fig. 1, show reduced solubilities in all the treated samples. Of enamel samples washed with 1/25, 1/100, 1/1000 and 1/10,000 sodium fluoride solutions, averages of 13.2,

* Carnegie Dental Fellow.

1 Dean, H. T., Jay, P., Arnold, F. A., McClure, F. J., and Elvove, E., *Public Health Reports*, 1939, **54**, 862.

2 Miller, B. F., Proc. Soc. Exp. Biol. and Med., 1938, **39**, 389.

3 Hodge, H. C., and Finn, S. B., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 318.

4 Armstrong, W. D., and Brekhus, P. J., *J. Dental Res.*, 1938, **17**, 393.

5 Smith, H. V., and Smith, M. C., *Waterworks Engineering*, 1937, **90**, 1600.

6 MacIntire, W. H., and Hammond, J. W., *Ind. Eng. Chem.*, 1938, **30**, 160.

7 Adler, H., Klein, G., and Lindsay, F. K., *Ind. Eng. Chem.*, 1938, **30**, 163.

8 Manly, R. S., and Hodge, H. C., *J. Dental Res.*, 1939, **18**, 133.

9 Volker, J., Thesis, University of Rochester, Rochester, N. Y., 1939.

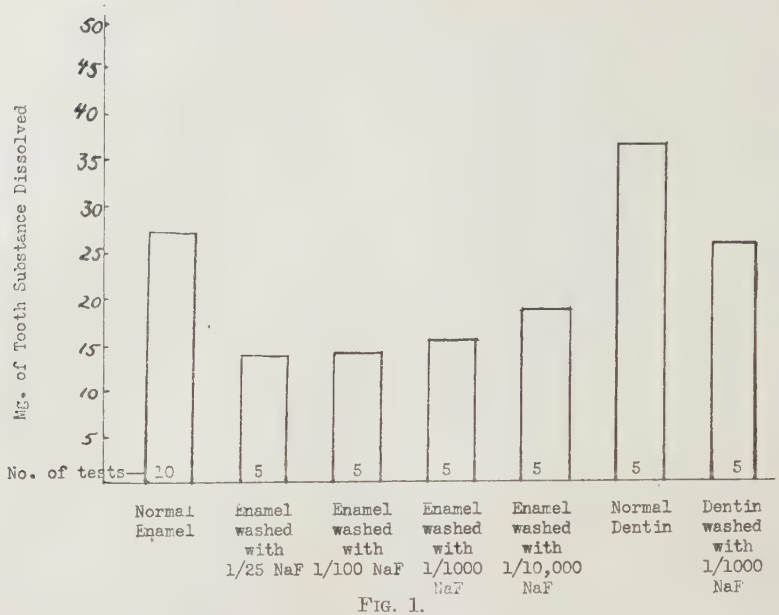

FIG. 1.
Average Solubilities of 50 mg Samples of Dental Hard Tissues.

14.1, 15.6 and 18.5 mg, respectively, dissolved as compared with an average of 27.3 mg for untreated samples. Normal dentin and dentin treated with 1/1000 sodium fluoride lost 36.1 and 26.0 mg, respectively. Confirmatory figures were obtained in 16 tests using decalcification periods of 5 and 30 minutes and 1½, 2 and 2½ hours. Five- to 10-minute applications of the fluoride were almost as effective in reducing the solubility as the one-hour treatments. The diminished solubility of the enamel was not lost after washing in water or saliva for periods up to 70 hours. It was also found that the natural surfaces of whole teeth treated with sodium fluoride were much less affected by acid than those of untreated teeth.

These observations seem to establish that fluorine reacts with tooth substance to produce a less soluble product. This reaction is probably similar to that occurring between fluorine and bone or other calcium phosphates and may consist of change to a fluorapatite, an adsorption of fluorine or a combination of both. The rapidity with which this reaction takes place makes it seem possible that, during the act of drinking, the fluorine from fluorine-containing water could combine to some extent with the teeth. Since such an effect would be most marked on the upper anterior teeth which would have the greatest contact with the water and on which there is a minimal amount of saliva, it is probably significant that Dean[1] found that the resistance to decay produced by fluorine-containing water was

definitely the most marked in these teeth. It is believed that these preliminary findings point to the use of controlled applications of fluorine-containing compounds as a means of preventing dental caries.

11030 P

The Fresh-Water Annelid, Tubifex, as a Pharmacological Test Object.

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A study of the regeneration of the fresh-water annelid, Tubifex, necessitated an evaluation of the factors conducing to its survival in the laboratory. Other workers[1, 2] have observed its satisfactory survival in tap-water. Our own experience has shown that it will not survive in the tap-water of New Orleans. The significant differences between the tap-water of New Orleans and the compatible tap-water of other cities appear to be the higher concentration of hydroxyl ion (pH 9-10) and the higher concentration of available chlorine of the water of this city. The chlorine content of New Orleans tap-water is stabilized at a minimum of 0.7 ppm by the addition of ammonium salts.

To investigate the influence of the reaction of the medium on the survival of Tubifex, solutions buffered with phosphate to cover the range from pH 6 to pH 8.4 were prepared. In the original solutions where the concentration of phosphate ion was 0.04 M there was no survival. The criterion of toxicity was death of all animals at the end of the arbitrarily chosen period of 18 hours. To ascertain at what concentrations of phosphate ion survival would result, a graded series of concentrations of phosphate ion for separate concentrations of hydroxyl ion from pH 4.6 to pH 8.4 were prepared. It was observed that Tubifex does survive in aqueous media if the phosphate ion concentration is low enough and also that the toxicity of phosphate ion is increased if the hydroxyl ion is increased. The toxicity seems to be a conjunctive result of phosphate ion with hydroxyl ion rather than an intrinsic toxicity of hydroxyl ion. This interpretation is strengthened by the survival of Tubifex in aqueous media from

1 Stone, R., *J. Morph.*, 1932, **53**, 389.

2 Stone, R., *J. Morph.*, 1933, **54**, 303.

which phosphate ion was excluded in the range pH 5.0 to 9.5. Boiled tap-water was shown to retain its alkaline reaction even when it was kept in contact with living animals as long as 18 hours, its final pH being 8.5.

The influence of residual active chlorine on the suitability of an aqueous medium for Tubifex was tested by subjecting the animals to varying concentrations of hypochlorous acid at pH 5.5. At concentrations of elementary chlorine (in hypochlorous acid) in excess of 0.6 ppm (0.0000085 molar) no worms survived. It should be pointed out that ordinary distilled water may contain appreciable quantities of active chlorine.[3]

The possibility that the chloramines,[4] produced in tap-water by the addition of ammonium ion to stabilize the concentration of residual chlorine were either more or less toxic than hypochlorous acid was tested by subjecting the animals to concentrations of chloramines at the previously utilized pH 5.5. The lethal concentration of chloramines in conjunction with non-reacted chlorine was found to be identical with that of hypochlorous acid, 0.6 ppm. The toxicity of the component ions of the ammonium sulphate used for producing chloramines was also measured. The requirements of the experimental situations were such that it was never necessary to add more ammonium ion than one-fortieth of the lethal concentration for that ion nor more sulphate ion than one-seven-hundredth of the lethal concentration of sulphate ion. The lethal concentrations of ammonium ion as ammonia nitrogen and sulphate ion are 20 and 390 ppm, respectively. It should be observed that the chloramine present at the experimental pH was largely dichloramine.[4]

The significant implication of the foregoing experiments is the extreme sensitivity of Tubifex to small quantities of residual chlorine and also of phosphate ion. It is conceivable that related forms and possibly even tissue slices are similarly sensitive, perhaps not always to the degree of actual destruction of the test-object but certainly to the degree of a possible significant modification of experimental results. It would appear that it might be routinely desirable to eliminate chlorine from a presumably good sample of distilled water prepared from tap-water known to be high in chlorine or chloramine content.

The phosphate ion concentrations of the stations where these animals have been collected were always lower than those concentrations which have been shown to be compatible with survival of Tubifex even under more average conjunctive conditions of pH.

[3] Anderson, A. K., and Zipkin, I., *J. Lab. and Clin. Med.*, 1939, **24**, 1209.
[4] Chapin, R. M., *J. A. C. S.*, 1929, **51**, 2112.

11031 P

Concentrating Capacity of the Kidney as Revealed by Injection of Posterior Pituitary Extract.*

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Although it has not been clearly established that there is a "ceiling concentration" representing the maximum concentrating capacity of the kidney tubules,[1] the ability of the kidney to secrete a concentrated urine is used as a clinical test of the functional capacity of this organ. The effect of posterior pituitary extract in retarding the excretion of water and at the same time increasing the output of solids is well known. It has been shown by Nelson and Woods that in mice the increase in solids (chlorides) may be great enough to break through the inhibitory effect on the elimination of water.[2] This simultaneous reduction in water and increase in chloride output might at some point give a urine which represented the maximum concentration possible for the given conditions. The present report presents the results obtained when the degree of concentration obtained by moderate restriction of food and water is compared with the concentration resulting from such restriction combined with the effect of posterior pituitary extract.

Experimental: Bladder fistula dogs were trained to stand in stocks for 3-hour collection periods. Prior to use food and water were withdrawn for 18 hours. Collections were made at 10-minute intervals while the animals were in the stocks. Urine concentrations were determined by specific gravity determinations (weighing in a 1 cc pyknometer), and by chloride determinations (using a modified Volhard-Arnold method). When posterior pituitary extract was given, one international unit as contained in a commercial preparation was given intramuscularly, in a volume of one cubic centimeter. Smith has commented adversely on the use of a mixture of pressor and antidiuretic hormones in such studies,[1] but we are not aware of satisfactory evidence against the generally accepted view that the

* This work was supported in part by Grant No. 315 of the Council on Pharmacy and Chemistry of the American Medical Association.

† The data are taken from the thesis presented by William Gosnell Paine in partial fulfillment of the requirements for the degree of Master of Science.

1 Smith, Homer W., *The Physiology of the Kidney*, Oxford Medical Publications, 1937.

2 Nelson, Erwin E., and Woods, G. G., *J. Pharm. Exp. Ther.*, 1934, **50**, 241.

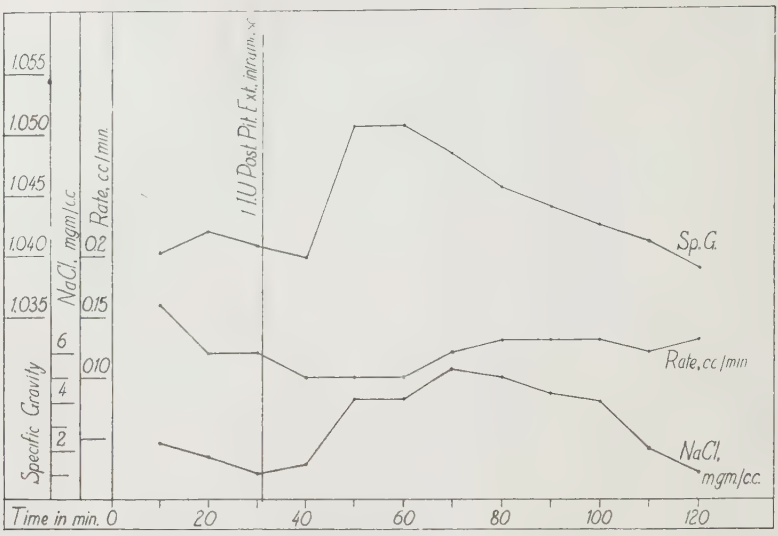

FIG. 1.

The effect of injection of 1 unit of posterior pituitary extract intramuscularly, on urine rate, urine chlorides and urine specific gravity, in a bladder fistula dog after 18 hours' withdrawal of food and water.

pressor and antidiuretic activities are resident in the same fraction.[3] Control experiments differed only in the failure to administer pituitary extract.

Fig. 1 gives the results of a typical experiment in which pituitary was given. Although the urine resulting from restriction was quite concentrated, (sp. gr. 1.043), after pituitary it rose in 20 minutes to appreciably higher values (sp. gr. 1.051). Similarly the chloride content increased from approximately 1 mg per cc to over 5 mg per cc. In different experiments the rate either rose slightly, remained constant, or as here fell slightly. It should be emphasized that until the animals have been adequately trained, it is difficult to get uniform rates of secretion during the control period. All experimental findings have been in the same direction as those shown in this figure. Ten controls in which only restriction was used, are in fact supplemented by the control periods prior to injection of pituitary in the 16 experiments in which this was given. The average rate in the controls was 0.11 cc per minute, specific gravity 1.038 and chloride content 2.2 mg per cc (as NaCl). After pituitary the average maximum specific gravity reached was 1.049 and the average maximum chloride 7 mg per cc. The rate changes have been mentioned. These chloride concentrations are by no means high, since in other

[3] Van Dyke, H. B., *The Physiology and Pharmacology of the Pituitary Body*, University of Chicago Press, 1936, p. 325.

experiments administration of extra chloride resulted in concentrations up to 20 mg per cc. Reference to the figure reveals that the specific gravity reached its maximum somewhat earlier than did the chlorides. This failure of the two peaks to coincide in time was a finding in every experiment. It may possibly be explained by the observation of Stehle that there is a greater increase in potassium than sodium in the urine formed under pituitary.[4] A change in the K:Na ratio would of course modify the specific gravity. Determinations of this ratio were not made. In a few experiments, however, it was found that the changes in urea concentration were not significant, and did not serve to explain the phenomenon.

Summary. In dogs, placed under conditions similar to those employed clinically for revealing the concentration capacity of the kidney, that is, under restriction of food and water, the administration of posterior pituitary extract results in the formation of a more concentrated urine than follows from restriction alone.

11032

Effect of Rabbit Adenocarcinoma Material on Brown-Pearce Rabbit Epithelioma.

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A material[1, 2, 3] which always has been present in primary and metastatic tissue obtained from the Brown-Pearce tumor of the rabbit has been shown to be filtrable through a Berkefeld "V" candle, desiccable, thermolabile (56°C) and different from the Duran-Reynals factor. It has consistently influenced the growth and spread of this particular strain of malignant cells *in vivo*, and when injected intratesticularly or intracutaneously into rabbits, in a dosage com-

4 Stehle, R. L., *Am. J. Physiol.*, 1927, **79**, 289.

1 Casey, A. E., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 816; 1933, **30**, 674, 1025; 1934, **31**, 663, 666; 1936, **34**, 111; 1939, **40**, 223, 228, 230; *Am. J. Cancer*, 1934, **21**, 760, 776; **22**, 665; 1936, **26**, 276; 1937, **31**, 446; *Arch. Path.*, 1935, **20**, 156; 1936, **22**, 275; 1937, **23**, 741; 1938, **25**, 754; 1939, **27**; *Proc. III Internat. Cancer Congress*, Atlantic City, Sept., 1939.

2 Casey, A. E., and Moragues, G. V., *Arch. Path.*, 1939, **27**; *Am. J. Cancer*, in press.

3 Erös, G., *Proc. III Internat. Cancer Congress*, Atlantic City, Sept., 1939.

parable to 0.0001-1.0 g of whole tumor tissue, 2 weeks before transplantation of the Brown-Pearce tumor, there results a greater incidence, volume and number of metastases, an increased mortality and a decreased longevity. Injection of the material into the rabbit evokes no demonstrable local or general reaction, and repeated injections have not rendered animals immune to transplantation of the same tumor.

Injection of the material into mice does not affect the growth and spread of Bashford carcinoma 63 or S180 of the mouse, nor have similarly prepared extracts of mouse tumors or of certain tumors of man, when injected into rabbits, affected the growth and spread of the Brown-Pearce tumor.

During the last few months, through the courtesy of Dr. H. S. N. Greene of the Rockefeller Institute (at Princeton), it has been possible to test the effects of a similarly prepared material from a transplantable uterine adenocarcinoma of the rabbit[4] on the growth and spread of the Brown-Pearce tumor. In this way it has been possible to test within the same species the effect of injection of a material of one carcinomatous origin on the growth and spread of a material of another carcinomatous origin.

Materials and Methods. The animal material consisted of 50 healthy New Zealand white male rabbits, 6 months of age.

Two sets of experiments were performed, in each of which 20 animals were subdivided into an experimental (Groups A and C) and a control (Groups B and D) group. As far as possible, each experimental animal was paired with a control litter mate.

The adenocarcinoma tissue, covered with paraffin and kept in a cold container, was sent by plane to New Orleans, where it was divided into 2 portions and kept in the freezing chamber of the ice box for 14 and 53 days respectively before being used. Ten animals (Group A) were injected with 0.5 cc of an approximately 5:1 dilution in normal saline of the material which had been refrigerated for 14 days, and 10 others (Group C) with the same amount of material which had been refrigerated for 53 days.

Two weeks after Groups A and C had been injected with the adenocarcinomatous material, all 40 animals (Groups A and C, and B and D) were injected intracutaneously over the left scapula with 0.5 cc of saline emulsion of Brown-Pearce tumor tissue.

Results. The primary tumors which had developed were measured in the animals in the first experiment (Groups A and B) 38 days and in the animals in the second experiment (Groups C and D)

[4] Greene, H. S. N., *J. Exp. Med.*, 1938, **67**, 691; 1939, **69**, 447.

33 days after transplantation of the Brown-Pearce tumor. At this time 14 of the 20 control animals had primary tumors which averaged 12.4 cm in volume ($\Sigma x = 173.8$; $\Sigma x^2 = 4631.32$) and 14 of the 20 experimental animals, also 70%, had primary tumors which averaged 12.8 cm in volume ($\Sigma x = 175.6$; $\Sigma x^2 = 3952.72$). These values for the incidence and volume of the primary tumors were not statistically different.

The experiments were terminated in Groups A and B 50 days after tumor inoculation, and in Groups C and D 55 days after tumor inoculation. At postmortem examination 14 of the 20 control animals (Groups B and D), 70%, were found to have primary tumors which by water displacement averaged 5.8 cc ($\Sigma x = 80.9$; $\Sigma x^2 = 1121.43$), and 15 of the 20 experimental animals (Groups A and B), 75%, were found to have primary tumors which averaged 4.6 cc ($\Sigma x = 68.3$; $\Sigma x^2 = 815.97$). There was no significant difference in the volume or in the incidence of the primary tumors in the two series at necropsy, and the average size of the primary tumors had greatly diminished in both the experimental and the control groups toward the latter part of the period of observation.

Nine animals in the experimental groups, 45%, and 9 in the control groups, 45%, were found at necropsy to have metastatic tumors which averaged 7.6 metastatic foci for each control animal ($\Sigma x = 68$; $\Sigma x^2 = 976$) and 2.8 metastatic foci for each experimental animal ($\Sigma x = 26$; $\Sigma x^2 = 132$). The volume of the metastatic tumors in each control animal averaged by water displacement 19.2 cc ($\Sigma x = 172.8$; $\Sigma x^2 = 9554.62$) and 3.1 cc in each experimental animal ($\Sigma x = 28.1$; $\Sigma x^2 = 180.21$).

There was thus no statistically significant difference in the incidence, volume or number of metastatic foci among the animals in the two series, regardless of whether the analysis was made on the basis of all the animals inoculated, on the basis of animals with primary tumors, or, finally, on the basis of animals with metastases. Since all the animals survived the 50-55 day period of observation, there was no difference in the actual mortality or longevity in the two series.

In earlier experiments it was shown that the injection 2 weeks before transplantation of material obtained from mouse tumors S180 and from Bashford carcinoma 63 promoted the growth of the homologous tumor but had no effect on heterologous tumors in the same species.[1]

Summary. Preliminary treatment with a material derived from a uterine adenocarcinoma of the rabbit failed to render rabbits more

susceptible to the subsequent transplantation of the Brown-Pearce tumor, and likewise failed to enhance its growth and spread. These results are in contrast to the marked enhancement in the incidence, growth and spread which constantly ensues when rabbits are treated with a material from the Brown-Pearce tumor 2 weeks before transplantation of that tumor.

11033 P

Respiratory Exchange of the Fresh Water Annelid, Tubifex.

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During an investigation to determine the availability of Tubifex tubifex as a test-object for certain pharmacological studies it was desired to define the magnitude of its respiratory exchange.

The method of Warburg[1] for measuring oxygen utilization (Q_{O_2}) and that of Dickens and Simer[2] for determining the respiratory quotient (R.Q.) were used. An aqueous medium containing NaCl—0.32%, KCl—0.007%, $CaCl_2$—0.00074%, and $NaHCO_3$—0.005%, possessing a pH range from 7.0 to 7.1, was used in the experiments. An atmosphere of oxygen, since it was found to be as suitable as air, was used for the sake of expediency in calculations. The most suitable temperature for these experiments was found to be 30°C; a few determinations were made at 25°C.

In most experiments preliminary anesthesia with chloretone (0.2%) was used to free the animals from mud and to sort them. Anesthesia lasting no more than 10 minutes was induced well in advance of the experiment. The data obtained from several comparative experiments indicate that such anesthesia induced long before the experiment did not modify appreciably the nature of the respiratory exchange.

The animals were arbitrarily divided into two categories based on length: small—about 2 cm; large—about 7 cm. No attempt to segregate the animals on a basis of sexual phase was made.

Utilization of Oxygen: In 12 determinations the average utilization of oxygen (Q_{O_2}) by small worms at 30°C was 1.49 cmm O_2

[1] Warburg, O., *Über den Stoffwechsel der Tumoren*, Berlin, 1926.

[2] Dickens, F., and Simer, F., *Biochem. J.*, 1931, **25**, 973.

per mg dry weight per hour when the animals were previously fasted for 16 hours. The average Q_{O_2} (4 determinations) for small worms previously fed glucose for 16 hours was 2.19. The experimental medium for the latter contained 0.2% glucose.

The addition of glucose to the experimental medium had little effect on the Q_{O_2} of small worms previously fasted 16 hours. The average Q_{O_2} (3 determinations) was 1.39.

The average Q_{O_2} of fasted small Tubifex at 25°C was 0.83 (9 determinations), whereas at 30°C it was 1.49 (12 determinations). In the case of large worms the average Q_{O_2} (9 determinations) at 25°C after a 16-hour fast was 0.80; at 30°C it was 1.03 (average of 10 determinations). The utilization of oxygen at 30°C by fasted small worms is in excess of that at 25°C by about 80%. This is greater than would be expected on a basis of the rule which postulates a two-fold increase in reaction velocity with a temperature rise of 10°C. In the case of the large worms the analogous increase was only about 30% and so is smaller than the increase expected because of the elevation in temperature. Furthermore, the utilization of oxygen by small worms is almost 45% higher at 30°C than that of large worms similarly treated; yet, at 25°C the difference is small.

The results are additionally of the nature of the average because during the determinations as many as 30 small worms or 15 large worms were placed in each of the experimental vessels.

Respiratory Quotient: An average of 4 determinations of the respiratory quotient (R.Q.) of small worms at 30°C fasted for 16 hours was 0.86. One of the values was abnormally high for some unaccounted reason and thus elevated the average. The average of the remaining 3 values was 0.81. When small worms were previously fed glucose for 16 hours and placed in an experimental medium containing 0.2% glucose their average R.Q. (6 determinations) was 0.89. In the case of small worms which were previously well fed, then fasted for 16 hours, and transferred to an experimental medium containing 0.2% glucose the average R.Q. (3 determinations) was 0.84.

The R.Q. of large worms fasted 16 hours and without glucose in the experimental vessels was 0.75 (average of 3 determinations). When glucose was added to the experimental medium the R.Q. was 0.81 (only one determination).

A comparison of the respiratory quotients leads one to suspect that a relatively short fasting period followed by the addition of glucose to the medium in the experimental vessel causes the R.Q. to be elevated only slightly. Prefeeding of the animals with glucose tends

to elevate the R.Q. The disparity between minima and maxima under similar experimental conditions is often great enough to indicate a difference in the quality of metabolism. This fact can be ascribed to a difference in antecedent nutrition of the individuals constituting the groups in separate experimental vessels. The magnitudes of the R.Q. in each case imply the conventional aerobic utilization of food substances.

Prefeeding of the animals with glucose in conjunction with the presence of glucose in the experimental vessels tends to increase Q_{O_2}. Analysis of the R.Q. values reveals, also, that this increase in the utilization of oxygen consequent to the accessibility of glucose is associated with a change in the nature of the R.Q.

The magnitude of oxygen uptake determined indirectly by the method of Dickens and Simer was satisfactorily comparable with that determined directly by the method of Warburg under analogous conditions.

11034 P

Conversion of Sulfanilamide into *p*-Hydroxylamino-benzenesulfonamide by Ultraviolet Irradiation.

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In a previous publication[1] the authors demonstrated that solutions of sulfanilamide and certain related compounds were endowed with anticatalase activity following irradiation with ultraviolet light. It was suggested in a subsequent paper[2] that this activity might result from the formation of either the *p*-hydroxylamino derivative or free hydroxylamine in the irradiated solutions. Taking hydroxylamine as a standard, it was calculated that the activity found could be explained by the conversion of about 2% of the sulfanilamide in an irradiated 8 mg % solution into the active compound. At that time no evidence was available to show that the assumed derivatives were actually formed under the conditions described. With a method recently developed by Rosenthal and Bauer,[3] it has been possible to

[1] Main, E. R., Shinn, L. E., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 272.

[2] Shinn, L. E., Main, E. R., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 591.

[3] Rosenthal, S. M., and Bauer, H., *Pub. Health Reports*, 1939, **54**, 1880.

demonstrate that solutions of sulfanilamide upon ultraviolet irradiation yield appreciable amounts of a substance which reacts as the *p*-hydroxylamino derivative.

Rosenthal and Bauer's method for the estimation of the *p*-hydroxylamino derivative in the presence of the free amine is based on the fact that in a mixture of the two substances, the amine can be acetylated by acetic anhydride and thus prevented from participating in the diazotization and coupling. The hydroxylamino form is not so affected and can thus be determined colorimetrically. As standards Rosenthal has recommended either *p*-hydroxylamino-benzene-sulfonamide or *p*-hydroxylaminobenzoic acid. We have chosen to use the benzoic acid.* Analyses were carried out precisely as in the specifications of Rosenthal and Bauer. The compounds were examined in simple, neutral aqueous solutions and in this respect were parallel to the standards. Complications arising from other organic materials which might be present in urine were, of course, not encountered.

Sulfanilamide was irradiated by exposing a thin layer of an aqueous solution for 1 min at a distance of 3 in. from an ultraviolet lamp. Irradiated and non-irradiated solutions were analyzed by the Rosenthal and Bauer method. Non-irradiated solutions gave a trace of color, but these colors were consistently too low for estimation. From the analyses the amount of material reacting as the *p*-hydroxylamino form was determined. The results are given in Table I.

It will be seen that under conditions comparable to those for which 2% conversion was postulated,[2] a compound reacting as the *p*-hydroxylamino form was found in a concentration of 0.19 mg %.

TABLE I.
Conversion of Sulfanilamide to the *p*-Hydroxylamino Derivative by Ultraviolet Irradiation.

A		B		
			Conc. of *p*-hydroxyl comp.	
Time sec	% converted	Conc. mg %	mg %	% converted
0	0	2	0.13	6.4
5	+	4	0.16	4.2
15	0.9	8	0.19	2.2
45	2.2	16	0.29	1.8
120	6.1	50	0.40	1.2
		100	+	+

A. Effect of duration of irradiation on an 8 mg % solution of sulfanilamide.
B. Effect of concentration of sulfanilamide solution at time of irradiation.

* Color matches were never perfect with the *p*-hydroxylamino benzoic acid as a standard but were not too difficult for adequately accurate colorimetric readings.

representing conversion of 2.2% of the original sulfanilamide. This supports the suggestion previously made that irradiated sulfanilamide solutions may owe their anticatalase activity to the presence of a hydroxylamino derivative. The presence of free hydroxylamine remains to be proven and the relative anticatalase activities of hydroxylamine and *p*-hydroxylamino-benzenesulfonamide is not at present known.

The method was applied to certain related compounds which had been examined for anticatalase activity. Non-irradiated solutions of 4,4'-diaminobenzene-sulfonanilide gave no measurable color when acetylated and diazotized. Irradiated solutions gave definite, measurable color. Sulfapyridine gave no color before or after irradiation. Three sulfones (methyl *p*-aminophenyl, *n*-amyl-*p*-aminophenyl and β-hydroxyethyl-*p*-aminophenyl sulfones) showed the failure of complete acetylation mentioned by Rosenthal and Bauer for other sulfones. The suggested double acetylation was not carried out. However, these sulfones did give stronger colors after irradiation, indicating a change similar to that in sulfanilamide.

11035 P

Experiments on the Antidermatitis Component of the Filtrate Factor in Rats.

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In rats fed a basal diet deficient in vitamin B and supplemented with vitamin B_1 and riboflavin, skin manifestations may persist or develop anew after the specific acrodynia has been moderated or cured by treatment with pure vitamin B_6 (natural or synthetic).[1, 2] Three more or less distinct types of lesions have been observed. In the most common type the lesions begin as sores around the mouth and as scaly dermatitis visible at first around the axillae, the groin and over the back between the scapulae. Later, alopecia follows and extends to the neck and over the back. In several animals, generalized scaliness (exfoliative dermatitis) has been observed.

[1] György, P., *J. Am. Chem. Soc.*, 1938, **60**, 983.

[2] György, P., and Eckardt, R. E., *Nature* (London), 1939, **144**, 512.

Administration of the proper doses of liver and yeast and of filtrates from extracts of wheat germ, yeast and rice polishings, which have been adsorbed on fuller's earth, assures complete cure of these skin manifestations. Thus, the factor curative of dermatitis in rats must be part of the filtrate factor (Factor 2).[3] The latter factor, as it appears to be a complex in itself, we prefer to designate the filtrate fraction.

With the production of a specific extensive dermatosis in rats that is due to lack of a part of the filtrate fraction, it becomes particularly interesting to know whether or not this component corresponds to the chick antidermatitis factor recently identified[4-7] with pantothenic acid.

Oleson, *et al.*,[8] were unable to reach a definite conclusion as to whether pantothenic acid was essential for the rat. Using growth as an unspecific criterion, Hoffer and Reichstein[9] stated that *beta*-alanine is active in promoting growth in rats deprived of the filtrate fraction, a finding which El-Sadr and his coworkers[10] were unable to confirm. In our experiments, *beta*-alanine in doses up to 500 micrograms daily had no effect on growth or on skin lesions.

Subbarow and Hitchings[11, 12] have prepared a crude calcium salt of pantothenic acid and were able to demonstrate definite growth-promoting activity in doses of 1 mg daily administered to rats fed a basal diet deficient in vitamin B and supplemented with vitamin B_1, riboflavin, and a fuller's earth adsorbate from liver extract as the source of vitamin B_6. Skin lesions were not observed.

Seemingly in contrast to these experiments, Ali Mohammad and his coworkers[13] reported lately that the growth-promoting activity of an iso-amyl alcohol extract from a rice bran preparation was not

3 Lepkovsky, S., Jukes, T. H., and Krause, M. E., *J. Biol. Chem.*, 1936, **115**, 557.

4 Jukes, T. H., *J. Am. Chem. Soc.*, 1939, **61**, 975.

5 Jukes, T. H., *J. Biol. Chem.*, 1939, **129**, 225.

6 Woolley, D. W., Waisman, H. A., and Elvehjem, C. A., *J. Am. Chem. Soc.*, 1939, **61**, 977.

7 Woolley, D. W., Waisman. H. A., and Elvehjem, C. A., *J. Biol. Chem.*, 1939, **129**, 673.

8 Oleson, J. J., Bird, H. R., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1939, **127**, 23.

9 Hoffer, M., and Reichstein, T., *Nature* (London), 1939, **144**, 72.

10 El-Sadr, M. M., Hind, H. G., Macrae, T. F., Work, C. E., Lythgoe, B., and Todd, A. R., *Nature* (London), 1939, **144**, 73.

11 Subbarow, Y., and Hitchings, G. H., *J. Am. Chem. Soc.*, 1939, **61**, 1615.

12 Hitchings, G. H., and Subbarow, Y., *J. Nutrition*, 1939, **18**, 265.

13 Mohammad, A., Emerson, O. H., Emerson, G. A., and Evans, H. M., *Science*, 1939, **90**, 377.

destroyed by heating in 1 *N* NaOH solution at 100°C for 1 hour and concluded that this factor is "not identical with the 'chick antidermatitis factor'."

As rats fed a diet devoid of the filtrate fraction exhibited specific skin symptoms which were at least comparable with those occurring in chicks, they were deemed to be suitable for testing an active concentrate containing the factor curative of dermatitis in rats. A zinc salt of pantothenic acid (containing other materials as listed below) was prepared by a slight modification of the method used by Hitchings and Subbarow.[12] This salt was tested on a sufficient number of rats (25 up to the present time) which were suffering from the specific skin lesions. The salt was composed of about 20% of zinc, 15% of pantothenic acid as determined by the streptococcus growth method, 30% of nicotinic acid amide, 10% of uracil, and the balance, about 25%, of unidentified material, mostly organic acids.

This preparation containing the zinc salt of pantothenic acid has been found very active in the cure, in from 3 to 4 weeks, of the specific skin lesions and in promotion of growth in the rats kept on a diet free from the filtrate fraction. The lowest active daily dose tested to date is 0.5 mg in terms of the zinc salt, which is equivalent to 75 micrograms of pantothenic acid. The zinc was precipitated by phosphate before it was administered. A fresh solution was prepared daily. Autoclaving at pH 10 for 2 hours at 120°C destroyed the activity of the salt.

Rats fed, in addition to their vitamin B-free basal diet, vitamin B_1, riboflavin, vitamin B_6 and the crude zinc salt of pantothenic acid appear still to manifest deficiency of other factors. Hepatic injury[14] was frequently observed in this group of animals.

The experiments here reported favor the view that pantothenic acid is efficacious in the cure of specific skin conditions in rats. A final conclusion requires repetition of the experiments, with a pure preparation of pantothenic acid.

Summary. A purified but still crude zinc salt of pantothenic acid proved to be active in the cure of specific skin lesions and in promotion of growth in rats fed a diet devoid of the filtrate fraction. Autoclaving at pH 10 destroyed the activity of the preparation.‡

[14] György, P., and Goldblatt, H., *J. Exp. Med.*, 1939, **70**, 185.

‡ Since this paper was submitted for publication, experiments with a crude but further purified barium salt containing 40 to 50% of pantothenic acid have shown it to be active in rats in a daily dose of 150 micrograms and over.

11036

Relative Spread of Particulate and Diffusible Substances in the Skin of Male and Female Rabbits.*

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From the Henry Phipps Institute, University of Pennsylvania, Philadelphia.

In an endeavor to elucidate the physiological basis for the varying inherited, natural resistance to tuberculosis demonstrated in certain inbred rabbit families,[1] it was found that among the factors determining this resistance, the spread of particulate matter in the skin played a prominent rôle.[2] In general, rabbits belonging to the susceptible families spread intracutaneously injected India ink over a wider area than rabbits belonging to the resistant strains. This observation was considered of significance, for the following reasons. In the resistant families tuberculosis naturally acquired by respiratory contagion, was limited to the lungs, and the rabbits died after a prolonged illness from a strictly localized ulcerative phthisis without spread to the draining lymph nodes and internal organs, a picture resembling in its essential characteristics the reinfection type of adult pulmonary tuberculosis in man. Rabbits of the susceptible families, on the other hand, died of a more rapidly fatal disease, originating in a primary focus in the lungs, soon spreading to the draining tracheobronchial lymph nodes, which became massively enlarged and caseous. Extensive caseous pneumonia without cavity formation developed forthwith and large nodular destructive lesions occurred in many organs from bacilli disseminated by the blood. The disease had the characteristics of a first infection and resembled the childhood type of tuberculosis in man.

There was thus a correspondence between the spread of carbon particles and the spread of tuberculosis in the tissues of these rabbit families.

It is well known that the mortality from tuberculosis is much greater in young women than in young men. In view of the parallel existing between the spread of India ink and resistance to tuberculosis, it seemed of interest to determine if sex differences can be observed experimentally in the spread of particulate matter. The

* This investigation was carried out as part of a cooperative study with the National Institute of Health of the United States Public Health Service.

† Technical assistant, U. S. Public Health Service.

1 Lurie, M. B., Proc. Soc. Exp. Biol. and Med., 1938, **39**, 176.

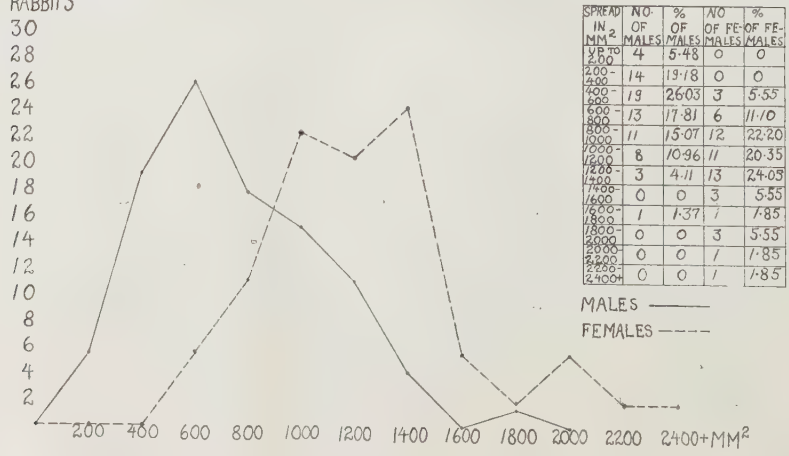
2 Lurie, M. B., *Ibid.*, 1938, **39**, 181.

spread of India ink was therefore studied in a series of male and female rabbits of the different families in our possession. Five-tenths of a cubic centimeter of an autoclaved 1:5 dilution of Higgins waterproof India ink in saline was injected intracutaneously in the flank. The approximate spread of the ink, in square millimeters, was determined on the next day by measuring with accurate calipers the 2 largest diameters of the stained area at right angles to each other. The product of these diameters was regarded as the area of spread. A total of 73 males and 54 females were tested. Seven rabbit families, each varying considerably in its capacity to spread India ink, were used. From 3 to 20 individuals of each sex from each family were examined. The age of the rabbits varied from 4 to 18 months. The mean age of the males was 7.2, with a standard deviation of the mean of 0.3. The mean age of the females was also 7.2 months with a standard deviation of the mean of 0.4. It is obvious that there was no difference in the age distribution of these males and females.

In Chart I are presented the data as well as the graphic curves of the distribution of the spread of India ink in the skin of these male and female rabbits. The mean spread in the males was 650 mm^2 with a standard deviation of the mean of ± 39. The mean spread in the females was 1155, with a standard deviation of the mean of ± 58. The critical ratio of the difference equals 7.7, which shows that this difference is beyond question statistically significant. This greater spreading power of the skin of females was evident in each of the 7 families tested, despite the very great range in the spread of India

CHART I.

THE SPREAD OF INDIA INK IN THE SKIN OF MALE AND FEMALE RABBITS ONE DAY AFTER INJECTION.



SPREAD IN MM^2	NO. OF MALES	% OF MALES	NO OF FEMALES	% OF FEMALES
UP TO 200	4	5·48	0	0
200–400	14	19·18	0	0
400–600	19	26·03	3	5·55
600–800	13	17·81	6	11·10
800–1000	11	15·07	12	22·20
1000–1200	8	10·96	11	20·35
1200–1400	3	4·11	13	24·05
1400–1600	0	0	3	5·55
1600–1800	1	1·37	1	1·85
1800–2000	0	0	3	5·55
2000–2200	0	0	1	1·85
2200–2400+	0	0	1	1·85

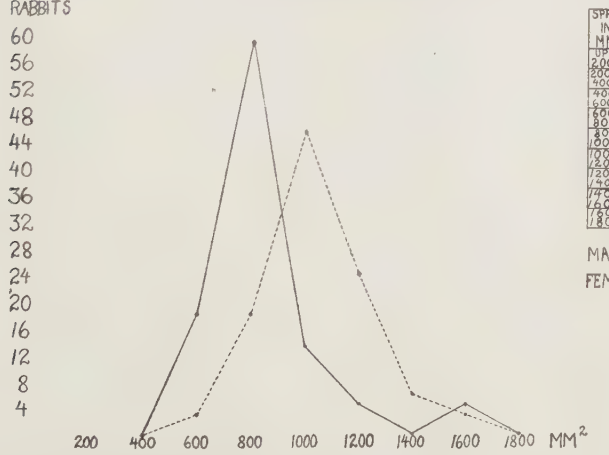
ink in the different families. For example, the average spread in the males of the resistant family "A" was 441 mm^2. The spread in "A" females was 819. Likewise the average spread of India ink in the susceptible "F" family was 720 for the males and 1077 for the females.

Similar determinations were made of the spread of a diffusible substance in the skin of many of the rabbits tested above with the particulate carbon The use of rabbit hemoglobin for this purpose was suggested by Madinaveitia.[3] The hemoglobin was prepared as outlined by this investigator and 0.3 cc was injected intracutaneously in the flank of the males and females. The spread of hemoglobin in the skin was determined as described above for India ink, 5 hours after the injection. At this time the margins of the hemoglobin-stained skin are very sharp. The next day, however, the margins are indistinct. Twenty-two males and 33 females were used. Members of all the 7 families previously tested with India ink were included. The age of the rabbits ranged from 4 to 18 months. The average age of the male rabbits was 8.9 with a standard deviation of the mean of 0.7. The average age of the female rabbits was 7.1 with a standard deviation of the mean of 0.6. The difference between the ages of the male and female rabbits was, however, not statistically significant.

In Chart II are presented the data as well as the graphic curves of the distribution of the spread of rabbit hemoglobin in the skin of

CHART II.

THE SPREAD OF HOMOLOGOUS HEMOGLOBIN IN THE SKIN OF MALE AND FEMALE RABBITS FIVE HOURS AFTER INJECTION.



SPREAD IN MM²	NO OF MALES	% OF MALES	NO OF FEMALES	% OF FEMALES
UP TO 200	0	0	0	0
200-400	0	0	0	0
400-600	4	18·16	1	3·03
600-800	13	59·02	6	18·18
800-1000	3	13·62	15	45·45
1000-1200	1	4·54	8	24·24
1200-1400	0	0	2	6·06
1400-1600	1	4·54	1	3·03
1600-1800	0	0	0	0

3 Madinaveitia, J., *Biochem. J.*, 1938, **32**, 1806.

these males and females. It is interesting to note that the range of spread of hemoglobin is much smaller than that of India ink. This is probably due to the shorter interval between the injection of the hemoglobin and the reading of the spread. Nevertheless, the mean spread of hemoglobin in the males was 728 mm^2, with a standard deviation of the mean of ± 47, while the average spread in the females was 937 with a standard deviation of the mean of ± 35. The critical ratio of the difference is 3.6, which places it in the realm of definite statistical significance. Not enough observations were obtained on each individual family to show a significant difference between the males and females of some families.

It is plain, therefore, that the spread of particulate and diffusible substances in the skin of rabbits is a sex limited character; the females spreading both to a greater degree. The age of the rabbits tested was in all instances above 3 months and reached up to 18 months. In this age range no difference was noted in the spreading capacity of younger and older rabbits.

The sex limited nature of the difference suggested trial of the effect of sex hormones on the spread of these substances. In a few experiments, the addition of as much as 50 international units of theelin to the standard dose of India ink or hemoglobin had no significant enhancing effect on their spread in the skin of male rabbits. Whether the greater spreading capacity of the skin of female rabbits for these substances is associated with a lower resistance to tuberculosis has not yet been determined.

11037 P

Oxygen Consumption of the Parts of the Amphibian Gastrula.*

L. G. BARTH.

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Interest in the organizer has led to the determination of its oxygen consumption and the results of various investigators do not agree. Brachet,[1] Brachet and Shapiro,[2] and Fischer and Hartwig,[3] agree that the dorsal lip exhibits a higher rate, while Waddington, Need-

* Aided by a grant from the Rockefeller Foundation.

1 Brachet, J., *Arch. de Biol.*, 1934, **46**, 25.

2 Brachet, J., and Shapiro, H., *J. Cell. Comp. Physiol.*, 1936, **10**, 133.

3 Fischer, F. G., and Hartwig, H., *Biol. Zent.*, 1938, **58**, 567.

ham and Brachet,[4] and Boell and Needham[5] report that the rates of O_2 consumption of the dorsal lip and ventral ectoderm are identical.

This seeming contradiction of results led to the present investigation. In one set of experiments the early gastrula was opened up and the roof of the blastocoel cut into 4 regions. Piece *1* contained the region just above the dorsal lip; *2* was composed chiefly of presumptive neural plate; *3* was chiefly presumptive epidermis, and *4* some epidermis and some cells which would give rise to the ventral lip. Numbers 1 and 4 were chosen carefully so as to contain about the same number of light-colored yolk cells and the regions were cut out so as to be in the same relative position to the equator of the gastrula. In some cases the oxygen consumption of the mass of large yolk-laden endoderm cells was measured.

In the second series of experiments the roof of the blastocoel was cut into 3 parts. Measurements of O_2 consumption were made by a modified Winklers method and the pieces dried and weighed on a micro balance. The results are given in mm^3 of O_2 per hr per 100 mg dry weight.

The results, Tables I and II, show clearly that a gradient in respiration is obtained on the ventral side of the gastrula ranging from 21.2 to 10.3 to 4.1. (Table I, *Rana pipiens* pieces 3, 4, 5). On the dorsal side, however, the respiration rate does not fall off so much with the result that the dorsal lip respires at almost the same rate as the presumptive epidermis. The dorsal lip consumes more oxygen than the ventral lip (Table I, pieces 1 and 4; Table II, pieces 1 and 3). The yolk-laden cells which give rise to endoderm respire at a very low rate.

Since there is a gradient in the amount of yolk in the cells of the gastrula, with the greatest amount present in the cells at the vegetal pole and least amount in the cells at the animal pole, it is not surprising that variable results are obtained when comparing the respiration of the dorsal lip with some other region of the gastrula. The dorsal

TABLE I.

Part extirpated	mm^3 of O_2/hr/100 mg dry weight		
	Ambystoma punctatum		*Rana pipiens*
1. dorsal lip	21.4	19.2	16.9
2. presumptive neural plate	23.8	20.4	28.6
3. presumptive epidermis	20.1	18.2	21.2
4. ventral lip	13.7	10.4	10.3
5. endoderm cells			4.1

[4] Waddington, C. H., Needham, J., and Brachet, J., *Proc. Roy. Soc. Lond.*, 1936, B **120**, 173.

[5] Boell, E. J., and Needham, J., *Proc. Roy. Soc. London*, 1939, B **127**, 363.

TABLE II.

Part extirpated	mm^3 of O_2/hr/100 mg dry weight			
	Ambystoma punctatum			*Rana pipiens*
1. dorsal lip	23.2	33.6	22.4	
		31.6	19.3	23.0
			18.5	
2. ectoderm	26.0	27.4	23.3	
		27.9	23.1	28.5
			28.0	
3. ventral lip	15.4	20.6	13.8	
		20.6	14.5	18.9
			13.8	
4. endoderm cells				7.7

lip forms in the vegetal half of the blastula where the cells contain larger amounts of yolk than in the animal half. This yolk does not respire and so determination of O_2 consumption will necessarily be low in this region. The O_2 consumption of the dorsal lip will then be greater than, equal to, or less than some other region of the gastrula, depending on the amount of yolk in the region which is used for comparison.

It is further clear that little or no interpretation can be placed upon the measurements until the gradient of yolk content is determined so that the oxygen consumption of the active protoplasm may be calculated. All that can be said is that the dorsal lip in spite of its yolk content exhibits a relatively high rate of O_2 consumption.

11038

Cystinuria in Two Scotch Terriers.

EDWIN R. BLAMEY AND ERWIN BRAND.

From the Laboratory of Dr. Edwin R. Blamey and from the Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York.

Cystinuria has been observed in the Dachshund[1] and more recently in a family of Irish terriers.[2-5] By inbreeding these Irish terriers, several animals with cystinuria have already been obtained.[5]

The present report deals with the occurrence of cystinuria in 2 *Scotch* terriers, Dogs Sc-1 and Sc-2. Since these dogs were pets, no extended observations could be made.

From one of these Scotch terriers (Sc-1) small calculi were removed, which had the typical appearance of cystine stones, and were identified as such by qualitative tests. Cystine crystals were present in the urine in considerable amounts. The analysis of a typical urine specimen is reported in Table I. The sample contained about 250 mg of cystine per liter, and the cystine sulfur accounted for about 20% of the total sulfur. Cystine was determined by the photometric method[6] as well as by the Sullivan[7] *cf.* [8] and Lugg-Sullivan[9] methods. The cystine values by the 3 methods are in close agreement, indicating the absence of substances which interfere with the Sullivan reaction.[3, 6] In general the sulfur partition and cystine excretion of this Scotch terrier resemble closely that of cystinuric Irish terrier No. 1.[3]

The second Scotch terrier (Sc-2) had no calculi, but cystine crystals could be identified in the urine. This dog excreted 200-400 mg of cystine per liter, and the sulfur distribution was typically cystinuric (Table I). However, the Sullivan reaction was negative in his urine. This does not indicate the absence of cystine, but rather the presence of interfering substances,* [3, 6] since after pre-

1 Lentz, W. J., *J. Am. Vet. Med. Assn.*, 1921, **59**, 365.

2 Morris, M. L., Green, D. F., Dinkel, J. H., and Brand, E., *North American Vet.*, 1935, **16**, 16.

3 Green, D. F., Morris, M. L., Cahill, G. F., and Brand, E., *J. Biol. Chem.*, 1936, **114**, 91.

4 Brand, E., and Cahill, G. F., *J. Biol. Chem.*, 1936, **114**, xv.

5 Brand, E., Cahill, G. F., and Slanetz, C. A., *J. Biol. Chem.*, 1938, **123**, xvi.

6 Kassell, B., and Brand, E., *J. Biol. Chem.*, 1938, **125**, 115.

7 Brand, E., Harris, M. M., and Biloon, S., *J. Biol. Chem.*, 1930, **86**, 315.

8 Kassell, B., and Brand, E., *J. Biol. Chem.*, 1938, **125**, 435.

9 Lugg, J. W. H., *Biochem. J.*, 1933, **27**, 668.

* The modification[10] of the Sullivan method, which eliminates to a considerable extent the influence of such interfering substances was not available at the time the urine of these Scotch terriers was investigated.

10 Sullivan, M. X., and Hess, W. C., *J. Biol. Chem.*, 1936, **116**, 221.

TABLE I.
Analysis of the Urine of Cystinuric Scotch Terriers.
Sulfur Distribution and Cystine Excretion.

Constituent	Dog Sc-1*			Dog Sc-2†			
				Sample 1			Sample 2
	g per liter	% of total S	% of neutral S	g per liter	% of total S	% of neutral S	g per liter
Total S	.38			.46			
Total SO_4	.20	53		.19	41		
Total neutral S	.18	47		.27	59		
Cystine S (photometric[6])	.07	19	46	.05	11	19	
Undetermined neutral S	.11	28	60	.24	48	81	
Cystine (photometric[6])	.27			.19			.45
" (Sullivan[7, 8])	.22			neg.			neg.
" (Lugg-Sullivan[9])	.24						
" (Cu_2Cl_2 precipitation followed by Sullivan method[6])							.35

* Cystine stones removed and cystine crystals in urine.
† Cystine crystals in urine.

cipitation of the cystine with cuprous chloride,[6] the values by the photometric and Sullivan methods are in reasonable agreement (Table I, Sc-2, Sample 2).

Summary. 1. Two cases of cystinuria in Scotch terriers have been described. One of them had cystine calculi. 2. Cystinuria with and without cystine calculi has been found in the following breeds of dogs: Dachshund, Irish terrier, Scotch terrier.

11039

Failure to Recover Pregnandiol Glucuronidate in Monkeys, Rabbits and Cats.*†

U. Westphal‡ and C. L. Buxton. (Introduced by E. T. Engle.)

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Previous experiments have shown that injected progesterone has been transformed into pregnandiol not only by women but also by men.[1] In pursuing this problem further, an attempt was made to find pregnandiol glucuronidate in the urine of experimental animals during normal cycles, during pregnancy and following injections of progesterone.

Urine was collected in metabolism cages and placed in the ice box with several drops of chloroform as a preservative. Examination for pregnandiol glucuronidate was then carried out on pooled specimens consisting of one or 2 liters of urine, according to the well proven method of Venning and Browne.[2] Urine from monkeys was collected from the first day of injection until 2 days after the last injection.

* Aided by a grant from the Rockefeller Foundation, administered by Dr. Philip E. Smith.

† The estradiol benzoate used was Progynon B, which was kindly furnished by Doctor Schwenk of Schering Corporation; the progesterone was Progestin, which was kindly furnished by Doctor Shaner of Roche-Organon, Inc.

‡ Fellow of the Rockefeller Foundation.

1 Buxton, C. L., and Westphal, U., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 284.

2 Venning, E. M., and Browne, J. S. L., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 792; Venning, E. M., *J. Biol. Chem.*, 1937, **119**, 473; Venning, E. M., *J. Biol. Chem.*, 1938, **126**, 595.

TABLE I.

Animal	No.	No. days collected and computed	Pregnandiol Glucuronidate
Rabbits			
Normal mature ♀	2	33	0
Pregnant ♀	2	28	0
Cats			
Normal mature ♀	1	40	0
Pregnant ♀	3	33	0

		Weight in g	Pretreatment with estradiol benzoate	Progesterone injected mg in days	
Adult monkeys					
Castrate ♀	1	4450	0	45 in 10	0
Castrate ♀	1	5700	0.4 mg in 10 d	50 in 10	0
Castrate ♀	1	4750	1.2 mg in 20 d	10 in 10	0
Normal ♂	1	5350	0	30 in 3	0
Normal ♂	1	5000	0	80 in 4	0

No pregnandiol glucuronidate was recovered in any instance. Also after hydrolysis no free pregnandiol was found in the monkey which received 80 mg of progesterone.

Table I shows the type and number of animals used.

11040

Vitamin E Deficiency in Dogs.*

H. D. ANDERSON, C. A. ELVEHJEM AND J. E. GONCE, JR. (Introduced by E. B. Hart.)

From the Departments of Biochemistry and Pediatrics, University of Wisconsin, Madison.

In a more extensive experiment designed to compare the nutritive values of raw, pasteurized and evaporated milks we have encountered an acute vitamin E deficiency in dogs. Since a deficiency of vitamin E has not been reported previously in this species we wish to take this opportunity to do so.

Weanling fox-terrier pups were fed a diet of a commercial evaporated milk (neither sweetened nor irradiated) which was diluted

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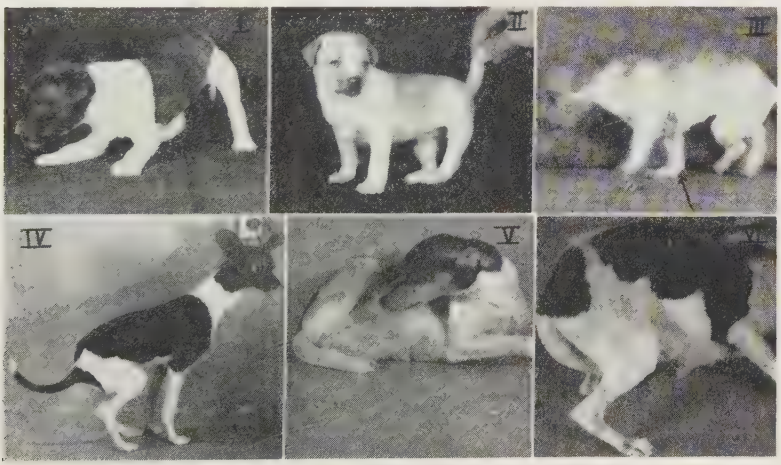
We are indebted to Hoffman-LaRoche, Inc., Nutley, New Jersey, for generous supplies of synthetic dl-α-tocopherol.

1:1 with water and placed in earthen containers suspended from the side of the pen to prevent waste and contamination. Minerals,† and cod liver oil‡ were added to the morning ration.

No sign of a nutritional deficiency appeared in these dogs except under the added strain of gestation and lactation. After 42 weeks female No. 1 became pregnant and produced a litter of 3 pups, one of which survived only a few hours, the others developed signs of muscle dystrophy about the twentieth day (Figs. I and II).

Faulty bone development was apparently not responsible for the condition since X-ray photographs showed no abnormalities. The greatest weakness appeared in the joint between the femur and tibia but, as shown in Fig. III, the muscles of the fore limb were also affected. A lack of muscle tonus in general and a hyper-sensitivity to pain seemed to be characteristic of the deficient animals. The marked denudation of the head and limbs, together with the dry and feverish skin suggested a cretin-like condition.

Various attempts were made to alleviate these symptoms. Subcutaneous injections of lysine and oral doses of liver extract powder



FIGS. I and IV, Dog No. 6. Note abnormal position of joint between femur and tibia.

FIG. II, Dog No. 7. Note crossing of hind legs as he walks, also flat-footed position of front foot.

FIGS. V and VI, Dog No. 1. Note lesions around teats and on the feet. Note also denudation on dog's head in Fig. V.

† Minerals were added at the rate of 5 mg of iron as the pyrophosphate, 1.0 mg of Cu as the sulfate, and 1.0 mg of Mn as the chloride per dog per day.

‡ Abbott's Cod Liver Oil administered at a level of 1 g per dog per day.

Vitamin D content $=>$ 100 U.S.P. units per g.

Vitamin A content $=>$ 1500 U.S.P. units per g.

had little or no effect, while wheat germ brought about a response in growth and an improvement in the general appearance of the dogs. No. 6 was then supplemented with dl-α-tocopherol and showed a marked improvement in vitality, muscle tonus and growth but continued administration of the crystalline vitamin over a period of 7 months has not cured the deformed posture as is shown by Fig. IV. It appeared then that we were dealing with a vitamin E deficiency but that the condition of muscle dystrophy was too far advanced to be cured.

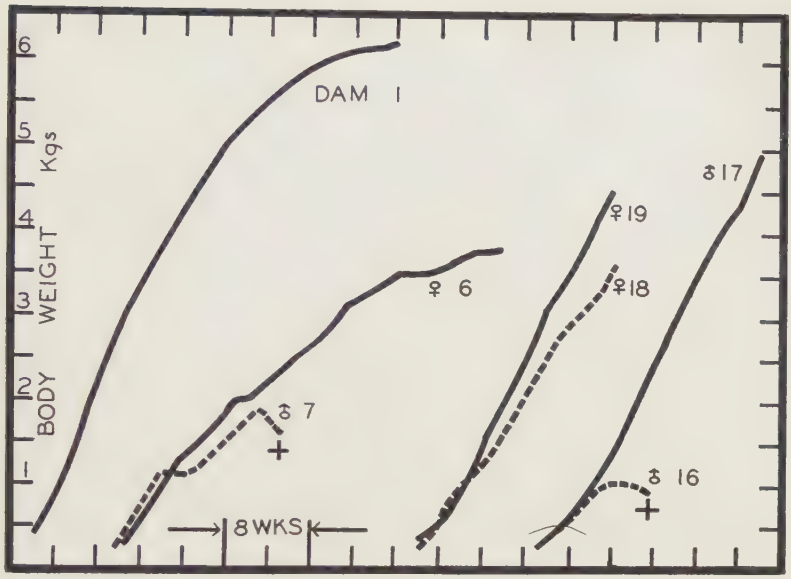
Pups of the second litter of female No. 1 likewise developed the early signs of dystrophy about the twentieth day. At this time, however, the dam also developed signs of a severe deficiency. Besides a marked loss in weight she developed sores on her teats, feet and other areas subjected to abrasion (Figs. V and VI). Her condition was such that it was thought advisable to remove the pups and raise them on the evaporated milk. Two of the pups, No. 17 and No. 19 received 5 mg of dl-α-tocopherol-acetate per week, while No. 16 and No. 18 were maintained as controls. It was apparent that the evaporated milk contained more vitamin E than the dam's milk, since the pups soon overcame the early symptoms of dystrophy.

The 2 pups (No. 17 and No. 19) which received the added vitamin E grew rapidly and, as is shown by the growth curves, these are the only ones which approximate the rapid growth of the original female dog. The pups which did not receive the added vitamin E grew much more slowly and the male pup No. 16 became so emaciated that he was sacrificed for histological examination although distinct muscle dystrophy had not set in.

The fact that in both litters the male dogs developed the deficiency symptoms earlier and to a greater degree indicates that the male dogs are more susceptible to this deficiency than are the females.

The general body appearance was distinctly different in the 2 groups of dogs. Those receiving the vitamin E supplement were sleek, fat pups with firm limbs and an abundance of vitality. Those receiving no vitamin E supplement became emaciated, with thin limbs and a marked weakness in their feet. Instead of standing firmly on their toes they sagged at the joints, often unable to keep their toes from curling (Fig. III).

Histological examination of the tissues showed a degeneration of the thyroid of dog No. 7, but no such changes in the thyroid of dog No. 16. The liver of dog No. 16 appeared to have undergone some hydropic changes and the Hassall bodies of the thyroid were very prominent. These differences may be partially explained by the rela-



GRAPH 1.

Growth curves: Dam 1—one of the original litter. No. 6 and No. 7—pups of dam 1—first litter—weaned by dam—developed muscle dystrophy about twentieth day. Nos. 16, 17, 18, and 19—pups of the second litter—transferred to evaporated milk on twentieth day—showed early signs of dystrophy—apparently recovered when transferred to evaporated milk. Nos. 17 and 19 received 5 mg vitamin E per week.

tive severity of the deficiency symptoms of the 2 dogs. No. 7 had developed the severe muscular paralysis, while No. 16 had shown some of the early signs of this paralysis but had recovered apparently when transferred to the evaporated milk, midway in the suckling period. Further studies on the other tissues have not been completed, but those which have been made, together with the gross symptoms and effects of supplementation point to the fact that this condition of paralysis is identical to the muscle dystrophy due to a vitamin E deficiency reported in other species.

Dam No. 1, when separated from her pups, was supplemented with 10 mg of vitamin E per week since the deficiency symptoms in the pups indicated that vitamin E might be the limiting factor.

Besides the open sores on her body, the skin on her belly appeared pale, wrinkled, cold to touch, and lifeless. There was a general denudation which was most prominent around the head and legs (Figs. V and VI). Eight weeks after the administration of the vitamin E these symptoms had disappeared, only slightly reddened areas remaining to indicate the position of former lesions.

Whether or not the vitamin E was the only limiting factor has not

been determined. Removal of the strain of lactation may have accounted for some of the effects. The answer to this problem will be forthcoming when she has produced another litter with the vitamin E supplied in suitable quantity.

Discussion. It has been clearly demonstrated that pups born to dogs on this ration of commercially evaporated milk will develop a vitamin E deficiency. This deficiency, if allowed to progress, will result in a muscular paralysis.

Nutritional muscle dystrophies have been reported in rats,[1] guinea pigs,[2] and rabbits,[3] on diets low in vitamin E. Attempts to produce similar conditions in dogs and cats,[3] on the diets used for rabbits, have apparently failed. Had these experiments with dogs and cats been continued long enough to include gestation and lactation, it is possible that the young would have developed muscle dystrophy.

Evidence has been produced[4] indicating that suckling rats born to E-low mothers *die,* not from lack of milk, but from a deficiency of vitamin E. If the factor were supplied or if the pups were transferred to normal mothers they survived. The secretion of vitamin E in human milk seems to be governed by the same factors since sterile E-low rats may be cured by feeding human milk,[5] providing the woman had been receiving a diet adequate in vitamin E.

These facts indicate that females conserve vitamin E for their own body needs at the expense of their suckling young. On a diet moderately low in this factor, it is reasonable to assume that the dam's milk would be still lower in this essential factor. Pups raised to weaning on such a milk would quite likely show more severe deficiency symptoms than pups which received the evaporated milk for half of their normal suckling period. Such was the case in this experiment but the change to the evaporated milk did not supply all of the essential factor as is shown by the growth response when dogs No. 17 and No. 19 received additional vitamin E.

Preliminary data indicate that raw milks may also be low enough in vitamin E to allow the development of similar conditions in the second generation pups. Thus the low supply of vitamin E may not be associated exclusively with milks that undergo processing. Since the diet solely of mineralized milk is an abnormal one for an adult dog the limiting amount of vitamin E may not be of great practical

1 Evans, H. M., and Burr, G. O., *J. Biol. Chem.*, 1938, **76**, 273.

2 Goettsch, M., and Pappenheimer, A. M., *J. Exp. Med.*, 1931, **54**, 145.

3 Morgulis, S., *Monographie Actualites Scientifiques et Industrielles*, Hermann and Cie, Paris, 1938, p. 74.

4 Barrie, M. M. O., *Nature*, 1937, **140**, 426.

5 Muller, C., *Schweitz Med. Wschr.*, 1936, **66**, 1164.

significance. The excellent performance of the dogs on mineralized milk until reproduction indicates that milk is adequate in vitamin E during the growing period for which milk is intended.

Summary. A deficiency in pups produced from dogs maintained for long periods of time on mineralized evaporated milk has been described. The condition is undoubtedly identical with muscle dystrophy previously described in rats, guinea pigs and rabbits and is cured by synthetic α-tocopherol if therapy is initiated before the symptoms are too far advanced.

11041 P

Infection of Guinea Pigs by Application of Virus of Lymphocytic Choriomeningitis to Their Normal Skins.

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The virus of lymphocytic choriomeningitis has been found to be infective for animals by a variety of routes[1-4] including, in addition to the more common ones, the intranasal,[2] intravaginal, intraurethral[5] and intrarectal.[6] Furthermore, it has been reported by Findlay and Stern[3] that, when this virus was fed to mice or applied to their lightly scarified skins, the mice did not exhibit apparent infection but the virus could be recovered from their spleens and kidneys. They also showed that when the virus was rubbed on the lightly scarified skins of 2 Rhesus monkeys, one showed a slight febrile reaction, the other no response. Recently, Shaughnessy and Milzer[7] demonstrated that the virus caused typical symptoms of the disease in guinea pigs when placed on their very lightly scarified skins.

The W. E. strain[8] of lymphocytic choriomeningitis virus was employed in these studies. Its virulence was such that, when 0.25 cc

1 Armstrong, C., and Lillie, E. D., *Pub. Health Rep.*, 1934, **49**, 1019.

2 Traub, E., *J. Exp. Med.*, 1936, **63**, 533.

3 Findlay, E. M., Alcock, N. S., and Stern, R. O., *Lancet*, 1936, **1**, 650.

4 Lepine, P., Kreis, B., and Sautter, V., *Compt. rend. Soc. biol.*, 1937, **124**, 420.

5 Wooley, J. S., Armstrong, C., and Onstott, R. H., *Pub. Health Rep.*, 1937, **52**, 1107.

6 Shaughnessy, H. J., and Zichis, J., unpublished studies.

7 Shaughnessy, H. J., and Milzer, A., *Am. J. Pub. Health*, 1937, **29**, 1103.

8 Scott, T. F. M., and Rivers, T. M., *J. Exp. Med.*, 1936, **63**, 397.

of a 1% suspension of an infected guinea pig brain was injected intracerebrally into guinea pigs, they showed signs of infection within 48 hours and died 6 to 8 days after exposure. In these experiments the inoculum consisted of a 10% suspension of infected guinea pig brain in heart infusion broth.

The guinea pigs and mice used were obtained from healthy stocks and it was demonstrated that they were not carriers of nor immune to the virus. The animals were also examined and found to be free of ecto-parasites.

The guinea pigs were exposed by placing from 0.5 cc to 1.2 cc of the virus suspension on the normal skin of the lateral dorso-lumbar region. The hairs were spread apart by means of the tip of a glass Luer syringe. At the same time the desired amount of virus was carefully deposited upon the skin from the same syringe without even touching the syringe to the skin. The inoculum did not dry to form a crust which might abrade the skin. The skin of the animals was examined with a hand lens for abrasions but none could be found. In order to minimize the chances of infection by the many possible means resulting from such exposure, screw-top screen capsules[9] were employed. The hair of the animals was not cut and the capsules were attached by means of an adhesive tape girdle. The virus was placed on the skin through the opening of the capsule, the cover of which was then replaced. This procedure was controlled by exposing animals in the same manner without the screen capsules. In addition, attempts were made to infect guinea pigs by spreading the virus suspension on their food and the litter in the cages.

The animals were kept in individual cages of solid construction with wire mesh covers. The temperature and condition of each animal were recorded daily. Following the observations of each animal and before observing the next animal, the operator thoroughly washed his gloved hands in a 20% solution of cresol compound, followed by washing with soap and water. Temperatures were taken of each animal with individual thermometers which were kept between observations in individual bottles containing 10% formalin. This technic was controlled by placing guinea pigs, to whose skins virus was not applied, between the exposed pigs and by subjecting them to the same technic of observation. In addition, the experiment was controlled by exposing groups of guinea pigs in rooms other than the one containing the intracerebrally inoculated virus control. Each group was observed and cared for by a different person.

In each experiment an identification of the virus was established

[9] Jellison, W. S., and Philip, C. B., *Pub. Health Rep.*, 1933, **48**, 1081.

by cultural studies, autopsy findings, transfer of the virus by subdural injection to Swiss mice, and in selected cases by neutralization tests with a specific immune serum.

Fifty guinea pigs were employed in this study. Thirty animals were exposed by placing a suspension of the virus on their normal skins. Of this number, screw-top capsules were attached to 18. Five pigs were inoculated intracerebrally to establish the virulence of the virus, and 10 were used as non-exposed controls. The remaining 5 animals were exposed by spreading the virus on the food and the litter in the cages.

Thirteen of the guinea pigs with the capsules died as a result of virus infection, 2 died of unknown causes and 3 survived without showing any clinical signs of infection. Of the 12 animals without the capsules, 8 died of virus infection and 4 survived without showing any apparent infection. The 5 pigs that were injected intracerebrally showed typical signs of infection and died. None of the control guinea pigs became infected.

In these experiments it was not possible to produce clinical infection in guinea pigs when the virus was spread on the food and litter of the cages. This was possibly due to less intimate contact of the virus with the skin, and to attenuation of the virus by exposure to drying and other physical factors. Our results are in general agreement with those of Traub.[10]

It is realized that minute abrasions, not visible with a hand lens, may have been present in the skins of these guinea pigs. However, any circumstances of this nature would be a factor encountered in any normal skin.

In view of these facts, it is believed that these results indicate that the virus of lymphocytic choriomeningitis may infect guinea pigs through the normal, apparently intact, skin.

[10] Traub, E., *J. Exp. Med.*, 1936, **63**, 183.

11042

Influence of Sex and Age on the Diabetic Traits in a Strain of Rats.*

Versa V. Cole and Ben K. Harned.

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Reports by the authors[1, 2] have shown that a high percentage of the sexually mature male rats from their "Y" strain exhibit characteristics of a hyper or unbalanced secretion of the diabetogenic factors of the anterior pituitary. Thus, their fasting blood sugar is high, their glucose tolerance is low, their hyperglycemic response to epinephrine is exaggerated, and their reaction to insulin is delayed. In the earlier work females were avoided, since they offered complicating factors not found in the males, but with the diabetic characteristics of the male established, it became necessary for genetic studies to investigate the female. Also, in view of the well-recognized sex differences both in metabolism and in the incidence of spontaneously occurring diseases[3–9] an investigation of the female appeared in order. The effect of age on the glucose tolerance of females has served to introduce an extension of the data on this factor in males.

The analytical methods, general procedure and criteria were the same as those used in studies on the male, and may be summarized as follows: Glucose tolerance tests on females from the "Y" and Wistar strains, maintained on the same diet and under the same conditions for 5 to 7 generations, were made 15 to 16 hours after food had been withdrawn. The glucose was administered intraperitoneally,† 3.5 g per kg, and followed by the determination of the

* This investigation was supported by a grant from the Committee on Research in Endocrinology, National Research Council.

1 Cole, V. V., and Harned, B. K., *Endocrinology*, 1938, **23**, 317.

2 Harned, B. K., and Cole, V. V., *Endocrinology*, 1939, **29**, 689.

3 Deuel, H. J., Hallman, L. F., and Murray, S., *J. Biol. Chem.*, 1937, **119**, 257.

4 Rose, M. S., and Hubbell, H. J., *J. Nutrition*, 1938, **15**, 91.

5 Lorenz, F. W., Entenman, C., and Chaikoff, I. L., *J. Biol. Chem.*, 1938, **122**, 619.

6 Evans, H. M., and Simpson, M. E., *Am. J. Physiol.*, 1931, **98**, 511.

7 Fitzhugh, O. G., *Am. J. Physiol.*, 1937, **118**, 677.

8 Ingle, D. J., *Proc. Staff Meet. Mayo Clinic*, 1938, **13**, 733.

9 Mosenthal, H. O., and Bolduan, C., *Am. J. Med. Sci.*, 1933, **186**, 605.

† A comparison of the results obtained by the intraperitoneal and intragastric administration of glucose was discussed in a previous paper.1

"true" blood sugar at appropriate intervals. The criteria for a normal curve demand that: (a) the half-hour blood sugar must be greater than the first hour value; (b) the half-hour value must be less than 300 mg %; (c) the fifth hour value must be at least 40 mg % below the half-hour or 60 mg % below if the half-hour value is over 200 mg %; (d) the fifth hour blood sugar must be below 180 mg %.

Data obtained with a large number of males from the authors' colony of the Wistar strain have failed to give a diabetic glucose tolerance in animals presenting a healthy physical appearance, and for this and other reasons[1, 2] the Wistar strain is considered normal. Tests on females of the normal strain (Table I) duplicate reasonably well those previously published on males,[1] however, the data on the females show a slightly larger probable error, and 2 of the 37 tests recorded in Table I are diabetic. Grouping the tests according to their period in the estrous cycle (Table I), both of the diabetic curves occur in diestrus, but when these tests were repeated during the same period of the cycle they were normal. The average of the tests made during diestrus is not significantly different from those in

TABLE I.

A Comparison of the Glucose Tolerance of Females from the Wistar and "Y" Strains.

Age range days	No. of rats	Fasting	½	1	2	3	5
		Hours after glucose*					
		"True" sugar in terms of glucose per 100 cc of blood					
		mg	mg	mg	mg	mg	mg
Wistar Strain							
114-141	18	69±1.1	172±3.0	125±3.5	110±1.5	108±2.2	105±3.8
134-148	19	72±1.1	187±3.9	119±2.8	102±1.9	101±2.0	96±2.8
Chance that deviation is due to sampling		19/100	41/100	39/100	9/100	11/100	19/100
Above tests grouped according to period in estrus cycle.							
	No. of tests						
Proestrus	9	7	70	161	120	107	103	101
Estrus and Metaestrus	14	8	68	171	120	104	103	98
Diestrus	14	8	73	186	126	107	106	102
"Y" Strain (Diabetic Curves)								
101-140		21	77	235	203	193	193	218
141-300		21	87	239	202	206	212	192
>300		12	77	216	188	211	225	250
"Y" Strain (Normal Curves)								
101-140		27	69	215	176	126	133	129
141-300		13	67	238	202	162	146	136
>300		7	79	219	192	160	150	140

* 3.5 g per kg in a 10% solution, intraperitoneally.

the other periods, nevertheless, this period was avoided in tests on the "Y" strain.

Tests on 101 females of the "Y" strain are reported in Table I, and comparisons of these data with those published on the males[1] and with those in Table III indicate no important sex differences;

TABLE II.
Statistical Significance of Age and Sex Differences in Incidence of Diabetic Curves. ("Y" Strain).

Sex	Age range	% diabetic	Compared with			Chance that deviation is due to sampling
			Sex	Age range	% diabetic	
Male	71-100	48	Male	51-70	14	.000,0007
			"	101-140	66	.02
			"	141-180	57	.27
			"	181-240	67	.018
			"	241-300	78	.0003
Male	101-140	66	"	141-180	57	.27
			"	181-240	67	.90
			"	241-300	78	.12
			Female	101-140	44	.014
			"	141-300	62	.70
			"	>300	62	.76

TABLE III.
Effect of Age on the Normal and Diabetic Glucose Tolerance Curves of Males from the "Y" Strain.

No. of rats	Age range days	Fasting	Hours after glucose* ½	1	2	3	5
		"True" sugar in terms of glucose per 100 cc of blood					
		mg	mg	mg	mg	mg	mg
		Diabetic Curves					
38	91-120	74	189	188	188	195	195
†61	121-180	78	199	204	197	201	202
43	181-240	78	188	182	177	185	186
†34	241-300	85	222	223	222	233	246
‡13	301-365	84	228	221	208	221	235
‡22	366-730	72	218	232	205	215	231
		Normal Curves					
20	91-120	76	210	195	161	146	136
40	121-180	77	210	186	155	151	133
21	181-240	83	223	205	164	159	141
10	241-300	75	217	194	157	151	135
‡3	301-365	82	212	206	166	149	155
‡2	366-730	81	220	213	179	177	115
†Chance that difference is due to sampling			9/1000	1/10	3/100	1/100	7/1000

* 3.5 g per kg in a 10% solution intraperitoneally.

‡ The ratios of diabetic to normal curves in the age groups over 300 days are grossly distorted because the normal rats have been sacrificed in other experiments.

the types of the diabetic curves are the same, the average values are of the same order, and the percentages of diabetic animals in the age groups are similar except for the group 101-140 days. Here only 44% of the females but 66% of the males are diabetic, and a statistical analysis of these data (Table II) shows that the chances are 100 to 1 that the difference is real. Thus, the females lag behind the males in the development of a low glucose tolerance and reach their maximum, 62% of diabetic curves, around 140 days of age. Attention should be called to the fact that the glucose tolerance of the males is normal to 50 days of age,[1] but thereafter the incidence of diabetic curves increases until it reaches 66% at 100 days of age with only a questionable additional increase after 240 days (Table II).

Relevant to the problem of whether or not the diabetic curves become more severe with age, Table III lists the averages for 307 tests made on males of the "Y" strain ranging in age from 90 to 730 days. The data suggest that after 240 days there is a further decrease in the glucose tolerance of the diabetic males. Normals of the "Y" strain do not show a similar change.

Summary. 1. Females of the "Y" strain exhibit a low glucose tolerance similar to that of the males. 2. The estrous cycle has no conspicuous effect on the glucose tolerance. 3. After 240 days of age, the severity of the diabetic curves in the males appears to increase.

11043 P

Androgenic Function of APL Stimulated Ovaries in Immature Rats.*

R. R. GREENE AND M. W. BURRILL. (Introduced by A. C. Ivy.)

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The present authors have reported that the administration of chorionic gonadotropic hormone (APL) to very young, immature female rats causes definite growth of the clitoris.[1] This gross enlargement was associated with modifications in the male direction as

* Supported in part by a grant from the Josiah Macy, Jr., Foundation. We wish to thank Dr. Charles Mellish and Dr. C. O. Miller of Lakeside Laboratories for the Anterior Pituitary-like Gonadotropic Hormone used in this study.

1 Greene, R. R., and Burrill, M. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 514.

shown by microscopical evidence. The ovary was indicated as being the probable source of the substance responsible for this androgenic activity. Bradbury and Gaensbauer[2] made similar observations and, in addition, noted that continuation of treatment beyond 30 days of age caused no further growth of the clitoris.

Inasmuch as clitorine growth is a relatively slow process and is not readily evaluated quantitatively, another method was devised for testing the production of androgens in the treated immature female. This method is based on two facts, (1) that the ventral prostate of the rat is very sensitive to androgens and (2) that histological evidence of androgenic stimulation of the prostate is definite and unequivocal. Although ventral prostatic lobes sometimes occur in normal female rats, the incidence is too low to be of practical value in approaching the present problem.[3, 4, 5] Furthermore, it has been shown that the ventral prostate of the immature female has a lower threshold to androgens than the prostate of the immature male, inasmuch as the ventral prostate of the normal immature female of a certain age shows evidence of activity when a male prostate implanted into the same female shows negative cytology.[5] The male prostate, therefore, by virtue of its higher threshold of response, when implanted into the treated immature female should provide a good indicator for the formation of androgenic substances in excess of the quantity normally produced by the immature female. The functional state of the implanted prostates should also determine whether or not the production of androgens by the immature female in response to treatment is definitely age-limited as indicated by Bradbury and Gaensbauer.[2]

Accordingly male ventral prostates were implanted intraperitoneally into 36 female rats at 10 days of age. The donors, in all cases, were litter mates of the recipients. These females were then given 25 to 100 RU of chorionic gonadotropin daily until they were sacrificed. Enlargement of the clitoris occurred as in previous experiments. The animals were killed at 15 to 38 days of age (5 to 28 days after implantation). Viable implants were recovered from 15 of them. The recovered tissue was fixed in Bouin's fluid, sectioned and studied microscopically. In the youngest prostate recovered (5 days post-implantation) the acinar epithelium was high but showed none of the

[2] Bradbury, Jas. T., and Gaensbauer, F., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 128.

[3] Witschi, E., Mahoney, J. J., and Riley, G. M., *Biol. Zentralb.*, 1938, **58**, 455.

[4] Price, Dorothy, PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 580.

[5] Burrill, M. W., and Greene, R. R., PROC. SOC. EXP. BIOL. AND MED., 1939. **42**, 764.

characteristic light areas which are indicative of androgenic stimulation.[6] The next age group included 7 animals, 18 to 25 days old. In 6 of these the implants were positive for androgenic function. The negative prostate was in an animal 23 days old. The oldest group included 7 animals, 28 to 38 days old. Only one implant in this group was positive. This was from one of 3 animals which were killed at 28 days of age.

In order to show that the ovary is necessary for the observed effects, 11 females were castrated at the time of implantation (10 days of age) and were then treated in the same manner as the preceding group. No clitorine enlargement was observed in these castrates. Viable implants were recovered from 8 of these animals at 20 to 35 days of age (6 at 25 days). None of these prostates showed evidence of androgenic stimulation.

Implants in the normal treated animals were a great deal larger than those observed in treated castrate controls. Due to the difficulty of making a clean dissection of the implanted tissues the prostates were not routinely weighed when recovered. However, several implants in the treated animals were over 30 mg and one, from a 25-day-old animal, weighed 60 mg. The average weight of the ventral prostates (both lobes) in the normal males of our colony at 26 days is 29.23 mg.

From these data it is evident that chorionic gonadotropin stimulates the ovaries of young, immature female rats to produce androgens in amounts sufficient to cause enlargement of the clitoris and to elicit a functional response in the implanted male prostates. The absence of functional activity in the implant at 15 days of age is probably due to insufficient time allowed for the response (5 days). In the period between 18 and 25 days of age, the stimulated ovaries produced sufficient androgen to induce a functional state in the implanted prostates. The negative state of the implants in the older group (28 to 38 days) implies that the output of androgens by the stimulated ovaries at this age is no longer adequate to maintain the prostates. However, the conclusion that the ovaries are no longer producing androgens is not warranted. It is also possible that the ovaries of the older animals are producing sufficient estrogens to antagonize directly the effect of the androgens on the prostatic epithelium.

The exact nature of the androgen produced by these stimulated ovaries is not known. It is probably similar to the androgen produced by chorionic gonadotropin-stimulated ovaries in the guinea

[6] Moore, C. R., Price, D., and Gallagher, T. F., *Am. J. Anat.*, 1930, **45**, 71.

pig[7, 8] and also to the androgen produced by ovaries transplanted to the ears of untreated castrate mice[9, 10] and rats.[11] In a previous publication the present authors suggested that the androgen involved may be progesterone[1] since this substance has been shown to have androgenic potency in the rat.[12, 13] Experiments to test this hypothesis are in progress.

Summary. Ventral prostates from male littermates were implanted into female rats 10 days of age. Daily treatment with chorionic gonadotropin produced no evidence of androgenic stimulation in the prostates when the females were castrated. When the ovaries were not removed the prostates of the treated animals showed evidence of stimulation at 18 to 25 days, but not after 28 days of age.

11044 P

Androgen Production in Normal Intact and Castrate Immature Female Rats.*

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In the female white rat the homologue of the male ventral prostate is sometimes found. Witschi, Mahoney, and Riley[1] reported that in one strain of rats the incidence of female prostates was 8.8% and in another strain, of Wistar origin, it was 26.7%. They also found that, by selective breeding, the incidence of female prostates was increased to 77.3%. Price[2] has reported a low incidence (under 2%) for the rats of her colony. In our own colony, examination of 333 females has placed the incidence at 13.8%.

Contrary to the conditions in the male, the female prostate is not

7 Papanicolaou, G., and Falk, E. A., PROC. SOC. EXP. BIOL. AND MED., 1934, **21**, 750.

8 Papanicolaou, G., and Falk, E. A., *Science*, 1938, **87**, 238.

9 Hill, R. T., *Endocrin.*, 1937, **21**, 495.

10 Hill, R. T., *Endocrin.*, 1937, **21**, 633.

11 Deanesly, Ruth, *Proc. Roy. Soc.* (Series B), 1938, **126**, 122.

12 Lamar, J. K., *Anat. Rec.*, 1937, **70**, Suppl. p. 45.

13 Greene, R. R., Burrill, M. W., and Ivy, A. C., *Endocrin.*, 1939, **24**, 351.

* Supported in part by the Josiah Macy, Jr., Foundation.

1 Witschi, E., Mahoney, J. J., and Riley, G. M., *Biol. Zentralb.*, 1938, **58**, 455.

2 Price, Dorothy, PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 580.

always bilobed. One lobe, either the right or the left, may be present alone. Among the 46 cases of female prostates found in our colony, 17 had bilateral lobes and 29 had unilateral lobes. Of the latter, 25 had right lobes only and 4 had left lobes only. This greater frequency of right prostates in the female was also observed by Witschi and his coworkers and by Price. Among the bilateral female prostates found in our colony some showed inequality in the sizes of the two lobes. In all these cases the right lobe was larger than the left.

In a series of 17 ventral prostates from intact, immature females ranging in age from 20 to 36 days we found evidence of functional activity in 11, using presence of light areas in the acinar cells[3] as the criterion of function. Price[2] has shown that the female prostate develops in a manner which is essentially similar to that found in the young castrated male. Light areas (indicating secretory activity) appear in the female prostate at about 21 days and remain until about 40 days, after which the gland regresses as it does in the male castrate. Our observations, therefore, accord with those of Price on the functional state of the immature female prostate.

The fact that the prostates of the immature females show evidence of functional activity indicates that androgenic substances are being produced by the immature female. In order to locate the source of these androgens, it was decided to compare the state of the prostate in castrated and intact females. Because of the low incidence of female prostates in our colony the ventral prostates from littermate males were implanted into the females to serve as indicators of androgen production. The male prostates were implanted intraperitoneally into 36 intact and 36 castrated females at 10 days of age. The animals were killed at 20 to 35 days of age. Implants were recovered and ventral prostates, whenever present, were also removed. Both were fixed in Bouin's fluid, sectioned and studied microscopically. An interesting fact was brought out by this procedure. From the intact animals 16 implants were recovered. These were all nonfunctional. The 16 implants recovered from the castrate females were also negative. However, in 4 of the intact implanted females, the ventral prostates which were also present showed evidence of functional activity in spite of the negative condition of the implanted male prostates. The female prostate in the immature rat, therefore, appears to have a lower threshold of sensitivity to androgens than the male prostate as judged by the positive response of the female prostate and the negative response of the male prostate in the same

[3] Moore, C. R., Price, D., and Gallagher, T. F., *Am. J. Anat.*, 1930, **45**, 71.

animal. This finding differs from Price's observation in the adult rat[2] since she found (unpublished data) the female prostate to have a higher threshold of response. The age of the animal, however, may be a determining factor in the relative sensitivity of the male and female prostates.

Ventral prostates were also found in 10 castrated females, 6 from castrates with implants and 4 from an additional group of 20 castrates which had no implants. Four of these 10 ventral prostates showed some evidence of function, although the degree of activity was less than in the ventral prostates of the intact animals. This finding may not be considered significant because of the small number of cases, but nevertheless is indicative of some extra-ovarian source of androgens in the immature female.

In a previous publication the authors[4] have presented evidence that the prostate of the immature male castrate rat is maintained in a functional state by the adrenal. This andromimetic capacity of the male adrenal is evident only up to the age of 31 days, after which it diminishes, and is lost by 41 days.[5] Price[2] has suggested that the temporary functional state of the normal immature female prostate may also be due to the production of androgenic substances by the adrenal. The few cases which we have presented of functional activity in the prostates of castrate immature females tend to substantiate this hypothesis. Affirmation awaits further accumulation of data.

Summary. Ventral prostates from immature male rats were implanted into female litter mates, both intact and castrated, at 10 days of age. All implants were functionally negative when recovered 10 to 25 days later. Of the 27 female ventral prostates found in intact and castrated immature females, 15 showed some degree of functional activity. The data obtained indicate (a) that in the immature rat the female prostate has a lower threshold of response to androgens than the male prostate, and (b) that there is an extra-ovarian source of androgens in the immature female rat.

[4] Burrill, M. W., and Greene, R. R., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 327.

[5] Burrill, M. W., and Greene, R. R., *Endocrin.*, 1940, in press.

11045 P

A Pressor Substance Produced by Anaerobic Autolysis of Renal Cortex.

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Many attempts have been made to identify the postulated pressor substance that causes high arterial pressure in animals with partially constricted renal arteries. Most of the attention by investigators of this problem has been focused on renin.[1, 2] Recently a heat-stable pressor substance that passes through a collodion membrane has been found in autolyzed renal cortex.[8] In this communication we wish to present information about a pressor substance that is produced by the renal cortex under anaerobic but not under aerobic conditions.

There is suggestive evidence pointing to oxygen or the lack of oxygen as the important factor controlling the production of a pressor substance in the kidney.[3–6] To test this factor the following experiment was performed.

Methods. Under aseptic conditions the renal cortex of the normal dog was separated from the medulla. The tissues were sliced by hand with a razor into sections less than .5 mm thick. These were then incubated in 2 parts, by weight, of dog plasma or Krebs[7] bicarbonate buffer solution in an atmosphere of 95% N_2-5% CO_2 for from 24 to 46 hours. Cultures of these 5 preparations revealed contamination by *B. subtilis* in all portions of one. The fluid portion was then separated from the tissue by centrifuging and then filtering through 6 layers of cheese cloth. The filtrate was injected intravenously into normal, completely nephrectomized, and hypertensive dogs. The standard dose was 1 cc (equivalent to 0.3 g of tissue) per 5 lb of body weight. Systolic blood pressure was measured by

* This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation.

1 Tigerstedt and Bergnan, *Scand. Arch. Physiol.*, 1898, **8**, 223.

2 Helmer and Page, *J. Biol. Chem.*, 1939, **127**, 273.

3 Goldblatt, Lynch, Hanzal, and Summerville, *J. Exp. Med.*, 1934, **59**, 347.

4 Harrison, Blaloch, Mason, and Williams, *Arch. Int. Med.*, 1937, **60**, 1058.

5 Houssay and Taquini, *Compt. rend. Soc. de biol.*, 1938, **129**, 860.

6 Taquini, *Compt. rend. Soc. de biol.*, 1939, **130**, 459.

7 Krebs and Henseleit, *Hoppe-Seyless Z. f. Physiol. Chem.*, 1932, **210**, 33.

8 Williams, Harrison, and Mason, *Am. J. Med. Sci.*, 1938, **195**, 339.

palpation of a Van Leersum carotid loop or the dorsalic pedis artery peripheral to a Riva Roca pressure cuff.

Results. In 18 of 20 instances the fluid obtained from anaerobic autolysis of renal cortex caused a rise in systolic blood pressure from 30 to over 300 (estimated) mm of Hg. Eight of these tests were made with normal dogs. The systolic blood pressure rose in 6 instances from 30 to 155 mm of Hg and remained elevated from 2 to 21 minutes. In the other 2 instances the systolic blood pressure fell 5 and 46 mm respectively. Nine injections in dogs with experimental renal hypertension raised the systolic blood pressure immediately. The range of increase varied from 68 to 300 (estimated) mm of Hg and the pressure remained above the base line from 30 to over 100 minutes.

Four bilaterally nephrectomized dogs (2 days postoperative) showed an increase in systolic blood pressure in each of the animals varying from 40 to 105 mm of Hg. The elevation of blood pressure lasted from 10 to 123 minutes. One animal in which a relatively poor response was obtained had had a severe hemorrhage during the operation 2 days previously.

The intravenous injection of material obtained by anaerobic autolysis of *renal medulla* produced no significant change in the blood pressure of 2 normal, 3 nephrectomized dogs, and 1 hypertensive dog. In one nephrectomized animal a rise of 105 mm Hg for 2 minutes followed the injection. However, in this animal, the injection of the cortical substance caused a rise of 105 mm Hg for over 50 minutes.

In contrast, when oxygen was substituted for nitrogen in the preparation of the kidney autolysates no pressor substance was manifest. On the contrary a strong depressor effect was produced by injection of the cortical substance. A fall in the systolic blood pressure of 40 to 186 mm Hg occurred in 8 of 10 cases. The injection of material derived from oxygenated medulla tissue was followed by no change in the blood pressure.

Other properties of the pressor substance may be mentioned. The amount of pressor substance increases with prolongation of anaerobiasis. The injection of 2 preparations that had been incubated for 24 hours raised the blood pressure of 3 normal dogs—5, 40 and 80 mm respectively while the injection of 3 preparations incubated for 42 hours raised the systolic blood pressure of 4 normal dogs 50, and more than 150, 150 and 155 mm Hg respectively. The pressor substance passes through a collodion membrane (6% parlodion in alcohol; ether, 60:40 and 4% glacial acetic acid). The ultrafiltrate so

obtained is active after 20 minutes of boiling and after standing for 4 days exposed to air in an icebox at 5°C. When injected into a hypertensive dog this ultrafiltrate raised the systolic blood pressure over 300 mm Hg. The pressor effect occurs immediately after intravenous injection. Whether this substance is a pressor amine has not been ascertained.

That neither cells nor bacteria are involved in the production of this pressor substance is shown by the following observations. Two preparations, one from fresh dog, the other from fresh beef kidney, were made by grinding the cortex and medulla separately. These were suspended in 2 parts by weight of distilled water for 1 hour, made isotonic with NaCl, centrifuged, filtered through filter paper and then through Seitz filters. The cell-free sterile (cultured) filtrates were set up, under the same condition as described above for the tissues, for 3 days. Control portions of the filtrates were kept on ice and tested at the same time as the incubated filtrates. With both beef and dog renal medullary and cortical filtrates the pressor effects were the same as were obtained with autolysed tissue under the same conditions. The untreated cortical filtrate produced a renin-like response while the anaerobically incubated cortical filtrate produced a pressor effect similar to that described for the anaerobically autolysed cortex. The aerobically incubated cortical filtrate produced a strong depressor substance as did the aerobically autolysed cortex. No comparable effects were observed with the filtrates of the medulla.

Conclusion. The above experiments show that anaerobic autolysis of renal cortex or cell-free cortical extract of the dog produces a powerful heat stable pressor substance that passes through a collodion membrane. This substance is not obtained from kidney medulla that has been subjected to the same conditions. Oxygen inhibits the formation of the pressor substance. The pressor action is manifest immediately after intravenous administration and it is greater and more sustained in hypertensive than in normal dogs. Increased quantities of pressor substance are produced as the anaerobic period is prolonged. The pressor substance is formed just as well in a substrate-free medium as in plasma.

11046

Blood Pressure of Pregnant Rabbits and its Response to Pitressin.*

E. W. Page and Eric Ogden. (Introduced by J. M. D. Olmsted.)

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Schockaert and Lambillon[1] have demonstrated that women in the latter half of a normal pregnancy are relatively insensitive to the pressor action of the postpituitary hormone, and that the blood serum of such women, when mixed with postpituitary hormone has an inhibitory action on the pressor component as compared to the serum of non-pregnant women. These findings have been applied to the study of human eclampsia.[2] This reports an attempt to determine whether such a phenomenon may be demonstrated in the rabbit.

We have found no reports of blood pressure records throughout pregnancy except for the human being, and it was necessary therefore to determine whether normal pregnancy in the rabbit produced any significant alteration of blood pressure. At various intervals throughout pregnancy, a standard dose of pitressin was administered intravenously to the unanesthetized animal to obtain the pressor response.

The method of Grant and Rothschild[3] was used for measuring the blood pressure on the central artery of the ear of warm, unanesthetized female rabbits weighing 3 to 4 kg. In one major respect our technic differed from theirs. Measurements were made at intervals of 10 to 15 seconds rather than 1 or 2 minutes. This offered a necessary advantage in enabling us to follow the rather rapid changes of blood pressure which follow the injection of pitressin. By actual tests it was found that the reactive hyperemia resulting from rapid determinations caused a lowering of the blood pressure only on the second reading and not thereafter. Readings were made until 5 consecutive values were found within 4 mm. The mean of these 5 readings was recorded as the resting blood pressure.

Pitressin was injected into the marginal vein of the ear opposite from the blood pressure capsule. A dose of 250 milliunits (0.25 ml

*Supported by the John and Mary Markle Foundation.

1 Schockaert, J. A., and Lambillon, J., *Compt. rend. Soc. biol.*, 1936, **123**, 309.

2 Lambillon, J., *Revue Belge des Sciences Medicales*, Dixieme Annee N1, 1938, 1.

3 Grant, R. T., and Rothschild, P., *J. Physiol.*, 1934, **81**, 265.

of a solution containing 1 pressor unit of pitressin† to each milliliter) was selected because it was large enough to produce invariably a clear-cut pressor response without depressor effects. Immediately after injection, readings were made as fast as possible and the mean of the 3 highest readings was taken. The difference between this mean and the resting blood pressure was recorded as the pressor response. Tests with injections of saline, and with noises, pain, and other stimuli showed that the duration of the response with slowing of the heart from pitressin are adequate for differentiation between rises due to pituitary hormone and the immediate and transient rises of reflex origin.

A number of the animals were subjected during pregnancy to laparotomy and to surgical procedures on the aorta or various of its branches below the level of the renal arteries. In some cases, these operations resulted in death or premature delivery, but they did not consistently affect the blood pressure.

The observations are summarized in Table I. They show that the pregnant animals did not differ from the nonpregnant group with respect to their blood pressure or their response to pitressin.

Conclusions. As to the blood pressure, these findings are not unlike those reported for women.[4] The response to pitressin, on the other hand, is significant in that it is identical in pregnant and nonpregnant rabbits in contrast to the findings reported for women.

TABLE I.
Comparison of Blood Pressure and Pitressin Response in Pregnant and Non-Pregnant Rabbits.

	No.of observations	Mean	σ Distribution	No. of rabbits	Mean value
Non-pregnant blood pressure	102	87.6±1.23	12.47	17	84.8
Pregnant blood pressure	168	87.45±.99	12.77	16	86.1
Non-pregnant pitressin response	61	30.25±.77	6.01	14	27.1
Pregnant pitressin response	60	27.60±1.01	7.81	11	27.5

The first 3 columns are computed directly from all the observations made. The last column shows the means of the best available estimate of blood pressure for each rabbit in the group.

† Our thanks are due Dr. Oliver Kamm of Parke Davis Laboratories for a supply of pitressin.

[4] Jensen, J., *The Heart in Pregnancy*, St. Louis, C. V. Mosby Co., 1938, Chap. XII.

11047

A Geometrical Determination of the Extracellular Space in Muscle.

ALEXANDER SANDOW. (Introduced by Harry A. Charipper.)

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Fenn[1] and his collaborators have demonstrated that 14.5% of the volume of the freshly excised frog skeletal muscle is unique in that it contains all the chloride of the muscle. This part of the muscle has been called the chloride space and the inference has been drawn that it is identical with the extracellular spaces. The purpose of this note is to add to the existing evidence in favor of this inference a purely geometrical demonstration that the extracellular spaces may well comprise some 15% of the muscle volume.

Consider a muscle, such as the sartorius, made up of parallel cylindrical fibers of diameter D, and let us calculate r, the ratio of the volume of the fiber space to that of the total space. In any thin cross-sectional segment of the muscle r will be given by the ratio of the area of all the fiber cross-sections to that of the entire cross-section. Assuming a regular arrangement of the fibers along the whole length of the muscle, it is clear that r for the whole muscle will be known if the ratio of the areas for any cross-section can be determined. And this can be calculated if some assumptions are made as to the nature of the packing of the fibers.

We may assume two ideal limiting types of packing, A and B (Fig 1.) If the packing is the A-type, the number of fibers per $cm^2 = 1/D^2$, and since the cross-sectional area of a fiber $= \pi D^2/4$ cm^2, the total area of the fibers in a cm^2 of muscle cross-section $= \pi/4$ cm^2. Therefore, $r = \pi/4 = 0.785$. If the packing is assumed to be of the B-type, then since the number of fibers of diameter D, per unit area of cross-section, is $2/\sqrt{3} \times$ the number for the A-type, $r = 2/\sqrt{3} \times 0.785 = 0.906$. Now it seems reasonable to believe that neither of these two types of packing is found in the actual muscle to the exclusion of the other. Both are probably present. If we assume that both are equally prevalent then we must take as the true theoretical value of r the average of the two values calculated above. This turns out to be 0.846. Therefore the geometrically determined value of the extra-fiber space is 0.154, *i. e.*, 15.4%, of the volume of the entire muscle.

[1] Fenn, W. O., Cobb, D. M., and Marsh, B. S., *Am. J. Physiol.*, 1934, **110**, 261.

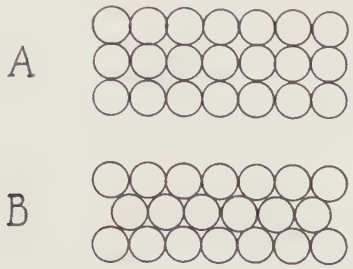

Fig. 1

Needless to say this is a highly simplified and idealized method of calculating the extracellular volume in muscle. No account, for example, has been taken of the effect of variation in the diameter of the fibers, nor of the effect of possible crowding and so distortion of the circular cross-section that has been assumed for the fiber. These and other factors, however, cannot be checked by reference to slides of actual cross-sections of muscle since the fibers suffer unpredictable alterations in the preparation of the sections. At any rate, the result obtained above is in striking agreement with Fenn's value of 14.5% for the chloride space, and in so far as the above considerations have validity they may be accepted as helping to confirm Fenn's conclusion as to the identity of the chloride space and the extracellular space in muscle.

11048

A Peculiar Reproductive Process in Colon Bacillus Colonies.*†

L. DIENES.

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In a previous publication attention was called to the fact that colonies resembling the L type colonies of *Streptobacillus moniliformis* develop in various bacterial cultures.[1] Observations to date

* The expenses of this investigation were defrayed in part by a grant from the Commonwealth Fund.

† This is publication No. 39 of the Lovett Memorial.

1 Dienes, L., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 636.

would seem to indicate that these L type of colonies originate from, or in close association with large swollen bacterial forms. If such observations have been interpreted correctly, then these large bacterial forms possess a definite biological significance. The occurrence of such large forms in bacterial cultures has been observed in the past. They have been regarded by the majority of bacteriologists as degenerative forms because they fail to multiply when transplanted.

In studying the L type of colonies, cultures of various bacterial strains were examined in the manner previously described *in situ* on the surface of the agar. If one examines such cultures from day to day one has an excellent opportunity of determining the fate of the large bodies. Our experience was similar to that of previous workers;[2] namely, that the large bodies disintegrate when transplanted without further development. During the course of this study, however, a single observation was made which seems sufficiently significant to warrant its being recorded.

Examination of a blood agar plate which had been inoculated with urinary sediment revealed large, intermediate, and tiny sized colonies. The larger colonies consisted of colon bacilli of the usual size and shape; whereas the tiny colonies were composed of long, wavy filaments showing all grades of transition into large fusiform or round bodies (Fig. 1). The large bodies were either uniformly stained or contained granules and vacuoles of different types. From Fig. 1 it is apparent that the large bacterial forms differ considerably from the normal appearance of colon bacilli. Such forms are present in small numbers in many freshly isolated colon bacillus cultures. In only 0.5% of the strains isolated from urine specimens did these forms appear in large numbers. These large forms are the result of the swelling of individual bacteria and bacterial filaments. They certainly do not represent the growth of an extraneous organism in the cultures. It was observed directly under the microscope that the filaments which produce the large bodies transferred on plain agar produce bacteria of normal shape. The study of transplants indicated also that the tiny colonies are genetically identical with the large colonies. The tiny colonies covered sufficiently large areas so as to render transplantation possible without contamination from the larger colonies. The first transplants on blood and ascitic agar plates again gave a mixture of tiny and large colonies. Subsequent trans-

[2] Klieneberger, E., *Ergebnisse der Hygiene, Bacteriologie, etc.*, 1930, **11**, 499, Springer, Berlin.

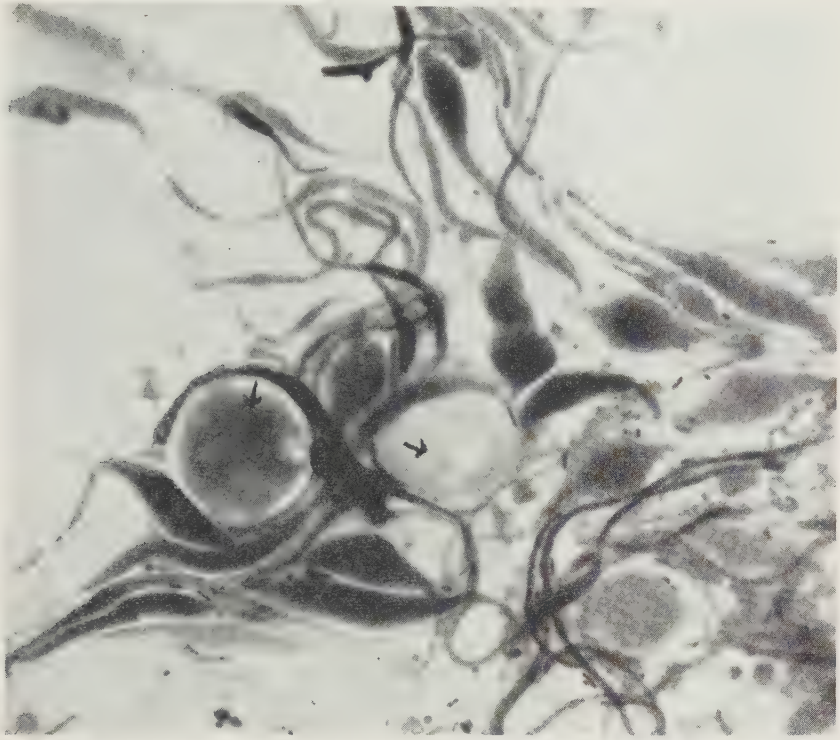

FIG. 1.

Fig. 1 represents a tiny colony showing transitory forms from filaments to large fusiform and round bodies. Two large bodies containing vacuoles are marked with arrows. Impression preparation from colonies stained with methylene blue on the surface of agar. (1:1000.)

plantation resulted in the appearance of normal appearing colonies. Transplants on plain agar produced normal sized colonies.

The original blood agar plate which had been inoculated with urinary sediment was retained and observed daily. During this period it was kept in the refrigerator except for several hours each day, when it was exposed to room temperature. Each day a small block of the agar was cut out, placed on a glass slide and stained with methylene blue. In the block removed on the sixth day 2 tiny colonies were seen which were different from the others. The large bodies in these 2 colonies appeared to be completely filled with small regular shaped bacteria. The membranes of these large bodies were intact and they differed from the large bodies in the other colonies only in the fact that their content was made up of bacteria. The whole colony consisted of these large round and fusiform bodies; bacterial growth outside of them was not present. The shape and appearance of these large bodies is very characteristic and cannot be

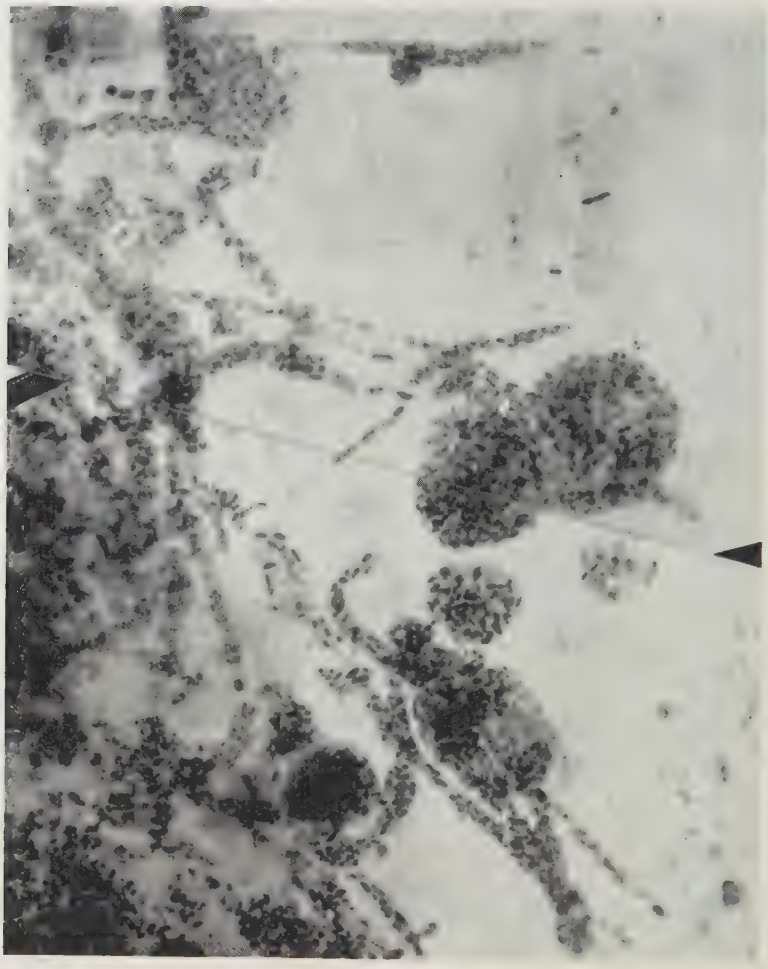

FIG. 2.

Fig. 2 represents a part of the colony in which the large bodies were filled with bacteria. Though the preparation was damaged it is apparent that the dense groups in which the bacteria are arranged imitate closely the shape of the large fusiform and round bodies. The whole colony consisted of similar structures. The fusiform body in the top center of the photograph shows in part the membrane which in the wet preparation surrounded all large bodies. (1:1000.)

mistaken for anything else. The preparation in this condition was seen by Dr. E. R. Sullivan and Dr. H. B. Arnold as well as by the author.

The making of a permanent preparation was only partially successful because the small agar block (approximately 3 x 6 mm) was crushed when the upper portion of the media containing the colonies was being cut off. In order to save the preparation the entire agar block was pressed into a thin layer on the slide and allowed to dry.

The membranes had disappeared in the dried preparation; however, the arrangement of the bacteria indicated clearly the shape of the large bodies. Fig. 2 is a photomicrograph of the dried preparation. Attention is called to the fact that the bacteria are arranged in the whole colony imitating the shape of the large bodies. Only in those places where the colony was injured during the preparation are the bacteria in formless masses.

It is interesting that all large bodies of the 2 adjacent colonies were filled with bacteria. The filaments from which the large bodies were produced were also fragmented into bacteria. Such transformations were never observed in thousands of these large bodies found in subsequent preparations made from the original plate and transplants. It is obvious that such transformation occurs rarely and then under conditions as yet unknown, but if these are fulfilled the transformation involves all the large bodies in a certain area of the culture.

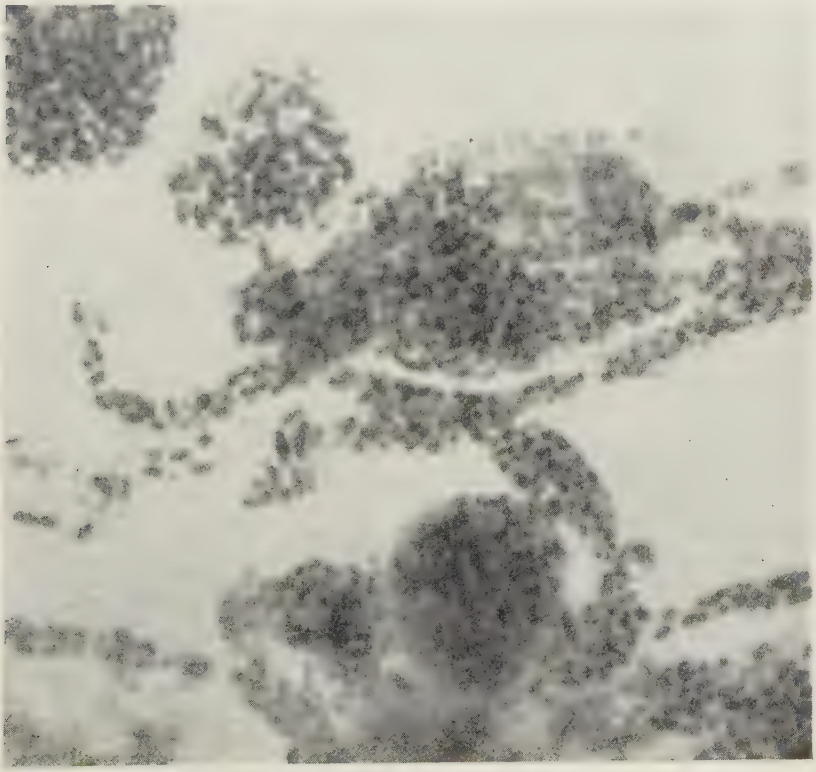

FIG. 3.

Fig. 3 gives details of Photograph 2 enlarged. In Fig. 3 the bacteria are plainly visible in the large bodies. (1:2000.)

The large bodies are very fragile structures and are readily destroyed by the slightest mechanical injury. It appears unlikely, therefore, that bacteria migrated into the large bodies from the outside and multiplied there without changing their shape. It would seem also unlikely that an accidental process such as the growing of bacteria into the large bodies would involve all large bodies of a single colony, including the bacterial filaments, and yet never occur in other colonies or in transplants. The most probable supposition to explain the presence of bacteria in the large bodies is that the bacteria were produced within them by transformation of their content.

The bacteria in the large bodies were of about the same size as normal colon bacilli. In some instances they were very small and coccoid in shape and many showed polar staining. In preparations made from the tiny colonies very small polar staining bacilli, round, deeply stained granules and transitional forms are seen frequently.

A previously recorded observation[3] may represent a process similar to that described in this paper. Reference is made to this observation with the purpose of pointing out that the staining properties of the large bodies filled with bacteria were similar to those observed in the present case. In wet preparations the large bodies were deeply stained and conspicuous. In dried preparations their membranes disappeared and their content could not be differentiated from other bacteria.

The observations described are well illustrated in the photomicrographs. From a study of these illustrations it would appear that the bacteria arranged in the shape of the large bodies were formed inside of such bodies. This was clearly visible in the original preparations. Similar observations have been described previously.‡ However, sufficient evidence was never presented to indicate that the forms seen in the large bodies were bacteria, or that the bacteria seen in the preparations originated from the large bodies.

The observation is presented here without further comment. These observations and those described previously suggest that the large bodies are not simply degeneration forms. It can be hoped that by the use of appropriate cultures and technical methods further information can be gained concerning the observed phenomena. The understanding of the nature and significance of the large bodies must await such further studies.

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[3] Dienes, L., *J. Inf. Diseases,* 1939, **65**, 24. (Reference is made to p. 40.)

‡ References may be found in the articles of Klieneberger,[2] Löhnis,[4]. Recently Nyberg[5] published similar observations.

[4] Löhnis, F., *National Academy of Science,* Vol. 16, 1921.

[5] Nyberg, C., *Zentralbl. f. Bakt.* (Abt. 1), 1938, **142**, 178; *Acta Path. Microbiol. Scand. Sup.,* 1938, **37**, 401.

11049 P

Effect of Testosterone Propionate on Creatinuria.*

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Numerous reports have been presented concerning the effects of castration, administration of androgens and estrogens in pure form, implantation of gonads, and injections of gonad extracts or saline suspensions upon creatine and creatinine metabolism in the normal and castrate animal. That a possible gonad relationship exists to creatine metabolism was shown by Rose[1] when he demonstrated a persistent creatinuria in children up to about the age of puberty. At this time creatinuria disappears in boys, but continues to a lesser or cyclic degree in girls and women. Read,[2] McNeal,[3] Remen,[4] Bühler,[5] and Pizzolato and Beard,[6] and others have presented evidence that castration in humans and animals leads to a creatinuria. In contrast, Tun-Chee-Shen,[7, 8, 9] Kochakian and Murlin,[10] Sandberg, Perla, and Holly,[11] and others have not observed an induced creatinuria in men, dogs, or rats by castration.

Considerable difference of opinion exists concerning the effects of sex hormones upon creatine and creatinine excretion in conditions of hypogonad function or castration. Bühler,[12, 13] Kun and Peczenik,[14] Paschkis and Schwoner,[15] and Kenyon, *et al.*,[16] find that androgens decrease hypogonad creatinuria. On the other hand, Pizzolato and Beard[6] claim that not only does castration in rats produce a creatin-

* These investigations were supported in part by a grant from the Rockefeller Foundation. We are indebted to Dr. E. Schwenk, Schering Corporation, Bloomfield, N. J., for the testosterone propionate.

1 Rose, W. C., *J. Biol. Chem.*, 1911, **10**, 265.

2 Read, B. E., *J. Biol. Chem.*, 1921, **47**, 281.

3 McNeal, M. D., *Am. J. Med. Sc.*, 1922, **144**, 222.

4 Remen, L., *Z. ges. exp. Med.*, 1932, **80**, 238.

5 Bühler, F., *Z. ges. exp. Med.*, 1935, **96**, 821.

6 Pizzolato, P., and Beard, H. H., *Endocrinology*, 1939, **24**, 358.

7 Tun-Chee-Shen, Proc. Soc. Exp. Biol. and Med., 1925, **22**, 408.

8 Tun-Chee-Shen, *Chinese J. Physiol.*, 1927, **1**, 363.

9 Tun-Chee-Shen and Hao Lin, *Chinese J. Physiol.*, 1927, **1**, 109.

10 Kochakian, C. D., and Murlin, J. R., *Am. J. Physiol.*, 1936, **117**, 642.

11 Sandberg, M., Perla, D., and Holly, O. M., *Endocrinology*, 1939, **24**, 503.

12 Bühler, F., *Z. ges. exp. Med.*, 1933, **86**, 650.

13 Bühler, F., *Z. ges. exp. Med.*, 1933, **86**, 638.

14 Kun, H., and Peczenik, O., *Arch. f. d. ges. Physiol.*, 1935, **236**, 471.

15 Paschkis, K., and Schwoner, A., *Arch. intern. pharmacodynamie*, 1936, **52**, 218.

16 Kenyon, A. T., *et al.*, in press.

TABLE I.

		Normal rats		Castrated rats	
	Day	Body wt, g	Creatine excreted, mg/kg body wt	Body wt, g	Creatine excreted, mg/kg body wt
Control period	2	563	0.9	488	1.2
	4	563	1.0	488	0.0
	6	563	0.5	488	0.0
	8	563	0.0	488	1.4
Fed creatine 40 mg/kg body wt	16	563	8.7	488	9.0
	18	557	12.0	488	12.0
	20	557	15.7	490	15.0
	22	557	11.0	490	14.7
	24	557	13.0	490	16.7
	26	557	15.5	490	20.0
	28	557	21.0	490	22.0
Fed creatine and injected testosterone propionate	30	557	20.7	490	21.2
	32	560	20.2	492	19.7
	34	566	20.0	512	18.0
	36	574	13.0	520	6.5
	38	588	13.7	538	2.7
	40	588	15.0	554	6.5
	42	588	18.7	548	15.0
Discontinued creatine and androgen	44	586	20.0	548	12.5
	48	578	11.0	544	1.0
	52	574	5.7	541	1.2
	56	568	0.5	538	1.5

uria but that testosterone propionate administration also increases it.

In view of the many conflicting reports and the realization of the difficulties attending the determination of creatine and creatinine, it was decided to study the quantitative determination of creatinine by means of the Jaffé reaction using the Evelyn photoelectric colorimeter and the Miller-Dubos specific enzyme for destroying creatine and creatinine. This was then applied to the study of the effect of testosterone propionate on exogenous creatine excretion in the normal and castrated adult male albino rat.

Sixteen male rats, all of the same age, were used. Eight of these were castrated at 3 months of age. All were placed in pairs in metabolism cages and kept in a constant temperature room which was maintained at 22°C. The urinary creatine investigations were divided into 4 periods: (1) a control period, (2) oral administration of creatine daily, (3) oral administration of creatine plus injection of 900 gamma of testosterone propionate daily, and (4) a period in which creatine and androgen were discontinued. The body weight and urinary creatine values of normal and castrated rats during these periods are shown in Table I.

These values for normal and castrated rats show the following: (1) the creatinuria in the control period is of a very low order; (2) as creatine is administered orally an intense creatinuria ensues; (3) as testosterone propionate and creatine are administered, there is produced a decrease in creatinuria, and a simultaneous increase in body weight which approximates a new high level. After this level is reached, creatine begins to appear again in greater quantities in the urine; and (4) in the fourth period where creatine and androgen were discontinued, there occurs a slight decrease in body weight and a return of creatine excretion to the pretreatment values. Therefore, the normal and castrate rats react in a similar fashion, but the changes in creatinuria and body weight are much greater in the castrate than in the normal animal.

The estimation of the creatine content of the gastrocnemius muscle in these rats with and without androgen administration, but always with a liberal supply of exogenous creatine showed no significant differences between the normal and castrated animals.

The gain in body weight of the animals is in accordance with the observations of many workers. Korenchevsky, Dennison, and Brovain[17] observed that the lower weight of castrated rats was elevated by injections of testosterone. Kenyon, Sandiford, Bryan, Knowlton, and Koch[18] have shown a definite weight increase in their eunuchoids during testosterone propionate administration. They feel that one-seventh to one-half of this gain may be due to protein being laid down as indicated by the nitrogen retention studies. They believe that a considerable amount of the weight increase is due to water and sodium retention. Similarly, Thorn and Harrop[19] have found that sodium and its associated water is retained in the normal dog during administration of estrone, estradiol, progesterone, pregnandiol, and testosterone, thus producing an increase in body weight.

Since it is generally believed that creatine when ingested is in part stored by the muscles, and that approximately 98% of the body's creatine resides in the musculature, it seems possible that the increased creatine retention, observed in the experiments described, paralleling body-weight gain, indicates increased muscle-tissue production under the influence of testosterone. This view is supported by the observation of Papanicolaou and Falk[20] who showed that the temporal muscles of male guinea pigs are larger than those of the females.

17 Korenchevsky, V., Dennison, M., and Brovain, I., *Biochem. J.*, 1936, **30**, 558.

18 Kenyon, A. T., Sandiford, I., Bryan, A. H., Knowlton, K., and Koch, F. C., *Endocrinology*, 1938, **23**, 135.

19 Thorn, G. W., and Harrop, G. H., *Science*, 1937, **86**, 40.

20 Papanicolaou, G. N., and Falk, E. A., *Science*, 1938, **87**, 238.

They also observed that in male and female castrates a muscular hypertrophy was produced by administration of testosterone.

It is, therefore, concluded that castration of adult male rats does not alter creatine excretion, that normal and castrated rats react in a similar fashion to exogenous creatine and testosterone propionate as far as creatine excretion and body weight changes are concerned, and that ingested creatine produces an intense creatinuria which is greatly inhibited by testosterone propionate administration.

11050

Changes in Excretion of Radioactive Na, K and in Carbohydrate Stores Twenty-four Hours following Adrenalectomy.*†

Evelyn Anderson, Michael Joseph and Virgil Herring. (Introduced by Herbert M. Evans.)

From the Institute of Experimental Biology and Department of Medicine, University of California, Berkeley and San Francisco.

Serious disturbances in electrolyte excretion and in carbohydrate storage have been observed in adrenal insufficiency. This investigation was undertaken with the hope of determining which of these two mechanisms first showed definite impairment. The findings presented here show that both conditions are altered in the rat 24 hours after the adrenals have been removed.

The altered rates of urinary excretion of radioactive sodium and potassium were interpreted as indicating altered excretion of these electrolytes. Evidence of alteration of carbohydrate metabolism was demonstrated by somewhat lowered values for blood sugar, and by lowered values for liver and muscle glycogen after glucose feeding. Separate groups of rats but of the same sex and approximately the same age, were used for these two studies, since it was not practicable to investigate both conditions in the same set of animals.

In the electrolyte excretion studies, male rats 10 weeks of age with an average weight of 268 g were used. The standardization of conditions for this experiment has been described previously.[1]

* We wish to acknowledge the assistance of the Federal Works Progress Administration, Project No. 10482-A5, and also the Christine Breon Fund.

† We wish to thank Dr. John A. Lawrence of the Radiation Laboratory for providing us with the radioactive isotopes used in this study.

1 Anderson, E., and Joseph, M., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 347.

Twenty-four hours before operation, the rats were placed in metabolism cages, and in place of the regular food and drink, they were given, by stomach tube, Locke's solution fortified with 6% glucose in amounts of 10 cc 3 times a day. Three groups of animals were used for the excretion studies of each electrolyte. In Group I both adrenals were removed; in Group II a "mock adrenalectomy" was performed, that is, the adrenals were exposed and handled, but not removed; in Group III no operation was performed. The operative work was done under ether anesthesia, and the anestheic period was not prolonged more than 10 minutes. Immediately following operation, the animals were given 1 cc of an isotonic solution of the radioactive isotope intraperitoneally. Thirty cc of Locke's solution with glucose was continued daily, and the urine was collected in 24-hour samples for 2 days. The amount of radioactive isotope excreted was measured by a Lauritzen electroscope.

In Table I are shown the urinary excretion rates for the radioactive sodium and potassium in the first 24 and 48 hour periods. This is expressed as the per cent of the total amount of radioactive isotope administered. In the first 24-hour period, the adrenalectomized rats showed a slight but significant increase in the excretion of Na^{24} over that of the mock adrenalectomized rats and the normal rats. In this same period the adrenalectomized rats showed a significant amount of K^{42} retention compared with the other 2 groups. The total excretion of Na^{24} and K^{42} for the first 48 hours after adrenalectomy showed the same general trend as in the 24-hour period.

For the study of carbohydrate storage, male rats 64-65 days of age were used. These animals were kept in a constant temperature chamber at 28° C during the experimental period, and for the 2 weeks preceding the experiment. During this time records were kept of the

TABLE I.
Changes in Urinary Excretion of Sodium^{24} and Potassium^{42} During 24- and 48-hour Periods After Adrenalectomy.

	Sodium^{24}			Potassium^{42}		
	No. animals	0-24 hr %*	0-48 hr %*	No. animals	0-24 hr %*	0-48 hr %*
Group I						
24 hr after adrenalectomy	6	16.3 (13.8-17.7)	35.0 (31.9-37.8)	10	2.9 (2.4-3.6)	4.9 (4.3-5.8)
Group II						
24 hr after "mock adrenalectomy"	3	13.4 (11.5-14.3)	30.2 (26.6-32.6)	10	4.5 (3.1-7.5)	9.1 (5.9-12.6)
Group III						
Normal	2	12.3 (11.2-13.4)	27.3 (27.3)	5	4.7 (3.1-5.0)	9.4 (8.7-10.4)

* % of the total amount of radioactive isotope administered.

TABLE II.
Changes in Carbohydrate Stores 24 Hours Following Adrenalectomy.

	No. animals	Body wt g	Glucose absorbed per 100 g body wt per hr	Blood sugar	Liver glycogen		Muscle glycogen, mg %
					mg %	mg per 100 g body wt	
Group I	7	195	128	115	471	15.9	389
24 hr after adrenalectomy			(94-177)*	(90-170)	(141-827)	(5.5-25.6)	(111-549)
Group II	7	196	145	155	1,148	42.0	606
24 hr after "mock adrenalectomy"			(133-172)	(115-185)	(768-1,492)	(28.2-56.5)	(53-941)
Group III	4	195	146	146	2,102	67.3	535
Normal			(134-165)	(120-180)	(1,498-2,862)	(53.3-78.3)	(191-753)
Group IV	3	184		88	62	2.2	395
Normal. Not fed†				(75-100)	(48-71)	(1.9-2.7)	(285-588)

* The figures in parentheses indicate the range of values.
† The total apparent glucose content of the gastrointestinal tract was 7.5 mg, 7.5 mg, and 10 mg respectively.

daily food intake, and the daily variation in body weight. At the time of operation which was 24 hours before sacrificing, the animals were divided into 4 groups carefully matched as to daily food intake and body weight. The adrenalectomized rats (Group I) had had an average daily food intake of 17.8 g for 3 days previously; the "mock adrenalectomized" rats (Group II) 17.8 g; the normal animals (Group III) 15.7 g, and the second group of normal animals (Group IV) 17.3 g. The animals of Groups I, II, and III were fasted 20 hours, given tap water to drink, and then fed 1.2 g glucose (5 cc of a 25% solution), by stomach tube, and sacrificed 4 hours later. Those of Group IV were fasted 24 hours, and sacrificed without feeding. The animals were anesthetized with sodium amytal, the tissues removed immediately, and the following determinations made: intestinal absorption of glucose; blood sugar; liver glycogen, and muscle glycogen. (Liver and muscle glycogen computations of Table II are expressed on a wet tissue weight.)

The results of the carbohydrate study are shown in Table II. After 24 hours the intestinal absorption of glucose is not significantly altered in the adrenalectomized animals as compared with the "mock adrenalectomized" and normal groups. On the other hand, the levels of blood sugar, liver glycogen, and muscle glycogen are significantly reduced 24 hours after adrenalectomy when compared with levels of normal animals. The "mock adrenalectomized" group also showed some lowering of the carbohydrate levels, although they were significantly higher than those of the adrenalectomized group.

It has not been feasible to repeat these 2 sets of experiments using a survival period of less than 24 hours after adrenalectomy. If a difference in time relationship of the onset of electrolyte and carbohydrate disturbances is to be detected, other experimental conditions will have to be set up. However, it appears highly significant that marked derangement in both the electrolyte and carbohydrate mechanism can be detected 24 hours after removal of the adrenals. From these observations one cannot make any assertions as to which disturbance is primary, and which secondary.

Summary. An increased urinary excretion of sodium, and a urinary retention of potassium are detectable in 24 hours after adrenalectomy. Simultaneous with these changes, there is found a lowered blood sugar, and lowered liver, and muscle glycogen values.

11051 P

Localization of Adrenal Cortical Hormones in the Adrenal Cortex of the Cat.

H. Stanley Bennett.* (Introduced by Joseph C. Aub.)

From the Department of Anatomy, Harvard Medical School, Boston.

Recently Reichstein and his collaborators (Reichstein,[1, 2] Reichstein and von Euw[3]) have isolated 3 ketone steroid compounds—corticosterone, desoxycorticosterone, and dehydrocorticosterone, any one of which will, in pure form, maintain the life of adrenalectomized animals. They have shown that all the biological activity in an alcoholic extract of beef adrenals is confined to the ketone fraction of the extract, whereas the ketone-free fraction is without specific activity in adrenalectomized animals (Reichstein[4]). All the active compounds so far isolated have been shown by Reichstein to possess 2 or 3 keto groups. They can be separated, with other keto compounds, from a crude extract by means of their reaction with semicarbazide, and they will reduce ammoniacal silver solution. This latter reaction is due to the presence in all three compounds of a side chain bearing an α-hydroxy keto grouping.

In the present study these reactions, with others, have been applied to frozen sections of fresh or formol-fixed cat adrenals in an attempt to localize the hormones in the cortex. The ascorbic acid was removed from the sections by treating with iodine, indophenol, or oxygenated glycine-sodium carbonate buffer at pH 8.4. The sections were then placed in a solution of phenylhydrazine hydrochloride in de-oxygenated sodium acetate-acetic acid buffer at pH 6.5-6 for a few hours. A distinct yellow band appeared in the outer portion of the fasciculata in the zone occupied by the large lipoid-rich cells called "spongiocytes" by Guieysse.[5] This yellow band appears to be due to the formation of phenylhydrazones with the carbonyl groups in the cortex. The phenylhydrazone formation was prevented by extracting the sections with acetone or alcohol, or by treating the sections with semicarbazide before immersing in phenylhydrazine. This zone

* This work was done while the author was a Fellow of the National Research Council, and was aided in part by a grant from the Josiah Macy, Jr., Foundation.

1 Reichstein, T., *Helv. Chim. Acta*, 1937, **20**, 953.

2 Reichstein, T., *Helv. Chim. Acta*, 1937, **20**, 978.

3 Reichstein, T., and von Euw, J., *Helv. Chim. Acta*, 1938, **21**, 1197.

4 Reichstein, T., *Helv. Chim. Acta*, 1936, **19**, 29.

5 Guieysse, A., *J. de l'anat. et de physiol.*, 1901, **37**, 312.

of cells was further characterized by reducing an ammoniacal silver solution. This reduction was also prevented by the previous treatment of the sections with acetone or alcohol or with semicarbazide. In cortical tissue outside this zone there was no detectable phenylhydrazone formation, or any reduction of ammoniacal silver solution which could be prevented by semicarbazide or extraction with acetone or alcohol.

It follows from these observations that the distribution of acetone or alcohol soluble keto compounds—and hence of the adrenal cortical hormones—must be confined to the zone of spongiocytes in the outer fasciculata, and that such compounds are absent in detectable quantities from other zones of the cat's adrenal cortex. Moreover, desoxycorticosterone acetate has been found to blacken osmic acid. From this it might be inferred that the hormones in the cells would also blacken osmic acid, and if this is so, the hormones must be present only in the osmophile portions of the cells showing the histochemical reactions consistent with those of the cortical hormones. This would localize the hormone more precisely in the osmophile lipoid vacuoles of the spongiocytes.

The zone of spongiocytes is further characterized by showing numerous birefringent crystals in frozen sections of glands treated with aqueous or alcoholic digitonin solution. These crystals are scanty or lacking in other zones of the cortex, and are probably for the most part due to the presence of cholesterol, which is of interest since Fieser[6] regards steroid hormones as probably being formed by the oxidation of cholesterol. Although Reichstein[2] regards this as highly questionable and thinks of cholesterol as perhaps an end-product of synthesis rather than a precursor of the hormone, the presence of cholesterol in the zone showing evidence of containing the hormone makes it seem likely that the adrenal secretion is actually formed in the spongiocytes and not merely stored there.

For these reasons the zone of spongiocytes, in which the adrenal cortical hormones and cholesterol are present, should be regarded as the "secretory zone" of the adrenal cortex of the cat.

Since it has been shown that the cells of the adrenal cortex form in the sub-capsular region and migrate toward the medulla, the zone of cells between the secretory zone and the capsule in which no phenylhydrazones form, and where osmophile vacuoles are scanty, could conveniently be termed the "presecretory zone," whereas the area between the secretory zone and the medulla could be regarded as

[6] Fieser, L. F., *The Chemistry of Natural Products Related to Phenanthrene*, Reinhold Publishing Co., 1936, p. 255.

the "postsecretory zone." The latter is in part characterized by the presence of numerous degenerating and senescent cells.

A complete account of the methods used and a correlation of the histochemical findings with histological and cytological data will appear in the *American Journal of Anatomy.*

11052

Determination of Prothrombin.

ARMAND J. QUICK.

From the Department of Pharmacology, Marquette University School of Medicine, Milwaukee, Wisconsin.

The author[1] developed a quantitative method for the determination of prothrombin based on the principle that the clotting time of blood or plasma is a quantitative measure of the prothrombin concentration provided an excess of thrombin and a constant concentration of calcium are present. For convenience and accuracy, the blood is oxalated and the test done on the plasma. It was demonstrated, however, that the test can be applied to whole blood.[2] In this determina-

TABLE I.

Comparison of Clotting Times of Unoxalated Plasma and of Recalcified Oxalated Plasma in Presence of Excess Thromboplastin.

	cc		cc
Chicken plasma unoxalated	0.1	Chicken plasma oxalated	0.1
Saline (0.85%)	0.1	Calcium chloride 0.025 M	0.1
Thromboplastin*	0.1	Thromboplastin*	0.1
Clotting time in seconds, 10 to 12		Clotting time in seconds, 10 to 11	
Goose plasma unoxalated	0.1	Goose plasma oxalated	0.1
Saline (0.85%)	0.1	Calcium chloride 0.025 M	0.1
Thromboplastin*	0.1	Thromboplastin*	0.1
Clotting time in seconds, 12		Clotting time in seconds, 11	
Human blood	0.9	Human plasma oxalated	0.1
Thromboplastin†	0.1	Calcium chloride 0.025 M	0.1
		Thromboplastin†	0.01
Clotting time in seconds, 12		Clotting time in seconds, 12½	

* From chicken brain.
† From rabbit brain.

[1] Quick, A. J., *J. Biol. Chem.*, 1935, **109**, lxxiii.

[2] Quick, A. J., Stanley-Brown, M., and Bancroft, F. W., *Am. J. Med. Sci.*, 1935, **190**, 501.

tion 0.1 cc of thromboplastin emulsion was added to 1 cc of blood obtained by venipuncture.

Theoretically there should be essentially no difference between the clotting time of recalcified oxalated plasma and unoxalated plasma or blood provided an excess of thromboplastin is present. This can be demonstrated experimentally as shown by the results of Table I.

Recently Smith and his associates[3] have adopted the author's method of determining the clotting time of 1 cc of blood containing 0.1 cc of thromboplastin as a "Bedside Test" for the determination of prothrombin. They employ the formula:

$$\text{Prothrombin activity (in \% of normal)} = \frac{\text{clotting time of normal blood}}{\text{clotting time of patient's blood}} \times 100$$

This formula is based on the assumption that the clotting time is a linear function of the concentration of prothrombin. The writer's quantitative studies of prothrombin in man,[4] in the rabbit, in the chicken[5] and other animals have shown that the relationship between the clotting time and the concentration of prothrombin is not linear. If the values are plotted, a hyperbolic curve is obtained which can be satisfactorily expressed by the equation:

$$\text{c.t.} = a + \frac{k}{c}$$

(c.t. = clotting time; c = concentration of prothrombin; a and k = constants.)[6]

For the exact quantitative determination of prothrombin, the author's test employing oxalated plasma has proved satisfactory in all experimental and clinical conditions, including the hemorrhagic disease of the newborn.[7] The difficulty of obtaining venous blood from young infants, however, has necessitated the development of a simple, roughly quantitative method for purely clinical purposes which is carried out as follows: A drop of blood obtained by a heel or ear lobe puncture is put on a glass slide, and mixed with a drop of equal size of thromboplastin. (Prepared according to the author's directions.)[4] The mixture is slowly stirred with a fine pointed stirring rod. By holding the glass slide over a light, the exact clotting time can readily be determined. Normal blood will clot in 15 to 20 seconds.

3 Smith, H. P., Ziffren, S. E., Owen, C. A., Hoffman, G. R., and Flynn, J. E., *J. Iowa Med. Soc.*, 1939, **29**, 377.

4 Quick, A. J., *J. A. M. A.*, 1938, **110**, 1658.

5 Quick, A. J., *Am. J. Physiol.*, 1936, **118**, 260.

6 Quick, A. J., and Leu, M., *J. Biol. Chem.*, 1937, **119**, lxxxi.

7 Quick, A. J., and Grossman, A. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 647; **41**, 227.

11053 P

Macromolecular Component of Chick Embryo Tissue Diseased with Western Strain Equine Encephalomyelitis Virus.*

D. Gordon Sharp, A. R. Taylor, Dorothy Beard, Harold Finkelstein and J. W. Beard.

From the Department of Surgery, Duke University School of Medicine, Durham, N. C.

A macromolecular component has been isolated[1] by ultracentrifugation from chick embryo tissue diseased with the Eastern strain of equine encephalomyelitis virus (E.S.). This protein, within the limits of tests thus far made, behaves as the virus and is specific[2] to the virus-diseased embryo tissue, though it is separable with difficulty from the lighter normal tissue component with $s_{20}^{\circ} = \text{ca } 70 \times 10^{-13}$ cm sec^{-1} dynes^{-1}.[3] In the present paper are described ultracentrifugal studies of extracts of chick embryo tissue diseased with Western strain virus (W.S.) from which a macromolecular protein possessing the properties of this strain has been isolated.

In a typical experiment, diseased tissue was extracted 24 hours at about 5°C in 4 times its volume of 0.15 M NaCl solution made to pH 8.5 with NH_4OH. Cleared of tissue debris by angle centrifugation, the extract was ultracentrifuged in 8 15-cc tubes at 67,000 g for 30 minutes. The 8 pellets were taken up in 60 cc water, pH 8.5 with NH_4OH, and the resulting suspension was spun in 4 tubes at 6000 g for 5 minutes. The supernatant fluid was then spun in 4 tubes at 17,000 g for 30 minutes. A specimen from the 4 pellets was dissolved in 0.2 M NaCl solution adjusted to pH 9.0 with NH_4OH for examination in the analytical ultracentrifuge (Fig. 1), and the remainder was taken up again in 60 cc water for repetition of the cycle of 6000 g and 17,000 g. The final pellets were dissolved in 0.2 M NaCl solution for ultracentrifugal analysis (Fig. 2).

The sedimentation diagram after the second cycle showed the indistinct, diffuse boundary (Fig. 1, a) of persisting $s_{20}^{\circ} = \text{ca } 70 \times 10^{-13}$ and the more prominent, somewhat diffuse boundary (Fig. 1, b) of a heavier material. After the third cycle, only the latter

* This work was aided by Lederle Laboratories, Pearl River, N. Y., and the ultracentrifuges were available through a grant from the National Cancer Institute.

[1] Wyckoff, R. W. G., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 771.

[2] Taylor, A. R., Sharp, D. G., Finkelstein, H., and Beard, J. W., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 462.

[3] Sharp, D. G., Taylor, A. R., Finkelstein, H., and Beard, J. W., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 459.

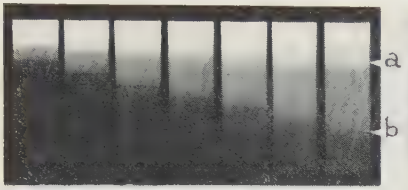

FIG. 1

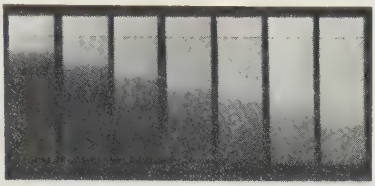

FIG. 2

The ultracentrifugal field employed to obtain the photographs was about 17,000 g and the interval between exposures was 3 minutes.

FIG. 1. Photograph of preparation obtained in the second fractionation cycle. The indistinct boundary at a indicates residual normal tissue component s_{20}° = ca 70 $\times$ 10^{-13} cm sec^{-1} $dynes^{-1}$ and the more prominent boundary b is that of the specific heavy component.

FIG. 2. The same preparation photographed after the third cycle. The boundary of only the heavier component with s_{20}° = ca 273 $\times$ 10^{-13} cm sec^{-1} $dynes^{-1}$ is present.

was seen (Fig. 2). The average constant for this material in third cycle pellets has been s_{20}° = ca 273 $\times$ 10^{-13} cm sec^{-1} $dynes^{-1}$.

The sedimentation constant of the W.S. protein, the identity of which was established by serological test, has been the same as that of the E.S. protein which, in our experience has given s_{20}° = ca 273 $\times$ 10^{-13} cm sec^{-1} $dynes^{-1}$. The W.S. protein is infectious to the order of 10^{13} mouse units per gram as compared with the order 10^{14} for E.S.[2] The yields of W.S. and E.S. proteins are similar, namely 0.5 to 1.0 mg per gram of tissue, indicating comparable concentrations in the respective diseased embryos.

In many respects the W.S. and E.S. proteins behave alike. Both can be obtained consistently free of contaminating salt-sensitive[2] s_{20}° =ca 70 $\times$ 10^{-13} by prolonging tissue extraction in 0.15 M NaCl solution for 1-3 days. The sharpest boundaries, however, are seen when fresh extracts are fractionated immediately, but here s_{20}° = ca 70 $\times$ 10^{-13} frequently persists through repeated cycles. Most of the inhomogeneous colloid regularly associated with the proteins can be eliminated also by careful fractionation, but preparations relatively free of it have always shown slightly diffuse boundaries.

In the experiment described here, water was used in the second and third fractionation cycles. Thus far we have been unable to photograph either W.S. or E.S. proteins in water while s_{20}° = ca 70 $\times$ 10^{-13} is seen regularly under these conditions. Either no boundary or a very diffuse shadow is seen where boundaries of the W.S. and E.S. proteins should appear. Pellets purified in saline and yielding sharp boundaries in saline show no boundary when the salt content is lowered to 0.05 M NaCl or less. This finding is a possible explanation of the high degree of infectivity associated with water

purified $s_{20}° = ca\ 70 \times 10^{-13}$ from diseased embryos.[2] In such water preparations the E.S. and W.S. proteins appear to be present but because of factors not yet clear, boundaries are not obtained.

11054

Specific Chemotherapy of Experimental Staphylococcus Infections with Thiazol Derivatives of Sulfanilamide.

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Experimental and clinical experience has shown that staphylococcus infections are either uninfluenced, or at best, slightly affected by sulfanilamide, sulfanilyl dimethyl sulfanilamide;[1, 2] by prontosil;[3] by p-Amino-benzene-sulphonyl-4-aminobenzenesulphonedimethylamide (uleron);[1, 2] di-(p-formylaminophenyl) sulphone and di-(p-butylaminophenyl) sulphone.[4] On the other hand, sulfanilyl sulfanilamide and sulfapyridine seemed to have some curative effects on experimental staphylococcus infections in mice.[2, 5]

The relative ineffectiveness of present chemotherapeutic compounds against staphylococcic infections stimulated researches for compounds of greater efficiency against this organism.

Three new thiazol derivatives of sulfanilamide have recently been developed. 2-sulfanilamidothiazol (sulfathiazol), 2-sulfanilamidomethylthiazol (sulfamethylthiazol), and 2-sulfanilamidophenylthiazol (sulfaphenylthiazol) are analogues of sulfapyridine.

Sulfathiazol and sulfamethylthiazol have since been described by Fosbinder and Walter.[6] Like sulfapyridine the thiazol compounds are equivalent to or superior to sulfanilamide against *Streptococcus hemolyticus* and the various strains of pneumococci, but unlike either of these preparations they exert a marked effect upon staphylococci, both *in vitro*[7] and *in vivo*.

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[1] Mellon, R. R., Shinn, L. E., and McBroom, J., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 563.

[2] Feinstone, W. H., Bliss, E. A., Ott, E., and Long, P. H., *Bull. Johns Hopkins Hosp.*, 1938, **62**, 565.

[3] Levaditi, C., and Vaisman, A., *Compt. rend. Soc. de biol.*, 1935, **119**, 946.

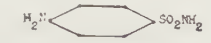
[4] Nitti, F., Bovet, D., and Hamon, V., *Compt. rend. Soc. de biol.*, 1938, **128**, 26.

[5] Whitby, L. E. H., *Lancet*, 1938, **2**, 1905.

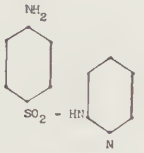
[6] Fosbinder, R. F., and Walter, L. A., *J. Am. Chem. Soc.*, 1939, **61**, 2033.

[7] to be presented elsewhere.

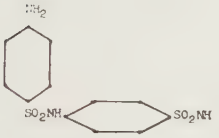
The structural formulae for sulfanilamide, sulfapyridine, sulfanilyl sulfanilamide and each of the thiazol derivatives are as follows:



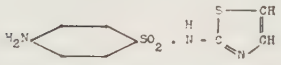
Sulfanilamide.



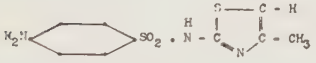
Sulfapyridine.



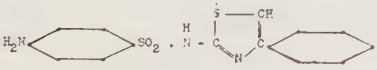
Sulfanilyl Sulfanilamide.



Sulfathiazol.



Sulfamethylthiazol.



Sulfaphenylthiazol.

This communication represents a preliminary comparison of the efficacy of the thiazol compounds with that of sulfapyridine and sulfanilyl sulfanilamide in experimental staphylococcus infections in mice.

Method. Male albino mice of uniform age from a standard strain weighing between 19 and 21 g were infected by the intravenous injection (tail vein) of 0.2 cc of a saline suspension of a highly mouse virulent strain of *Staphylococcus aureus* representing roughly 800 million cocci. This strain (F.D.A.209) was obtained from a human case and kept in stock culture. The heavily abscessed kidneys of the infected animals were removed at the time of death for reculture on agar slants. Such transfers (usually 4) were carried out in successive groups of mice until the virulence developed was such that the above infection uniformly killed 100% of a series of at least 10 mice within 1 to 5 days. The culture of such virulence was used in all chemotherapeutic tests.

TABLE I.
Chemotherapy of Experimental *Staphylococcus aureus* Infections in Mice.

Preparation	Single dose, g/kg	Total dose, g/kg	Died before 20th day	Day on which death occurred (individual animals)	Pathologic findings
None (controls)			10/10	1,2,3,3,4,4,4,4,5,5	++ to ++++ (influenced by time of survival)
Sulfapyridine	1.0	18.0	6/10	5,6,6,11,12,16	++ +++ ++++ ++++
Sulfanilyl sulfanilamide	1.0	18.0	7/10	2,5,6,6,7,8,15	++ +++ ++++
Sulfathiazol	1.0	18.0	3/10	1½,4,12	0 0 0 + ++ ++ ++++
Sulfamethylthiazol	1.0	18.0	1/10	10	0 0 0 + + ++ ++ ++ +++
Sulfaphenylthiazol	1.0	18.0	5/10	8,13,14,16,19	++ +++ ++++ ++++ ++++

Key: 0 Kidneys pale, ⅓ normal size, no active abscess. No abscess in urinary tract. No abscess in knee joint.
\+ Same as 0 except that there is one apparently active pin point abscess on one kidney.
++ Same as 0 except that there are 2 or 3 pin point abscesses on one kidney that look active, but still show healing.
+++ Three or more abscesses on one or both kidneys. No abscess in urinary or genital tract. One knee joint abscess.
++++ Numerous abscesses on both kidneys. Abscess in urinary tract. Knee joint abscess.

TABLE II.
Chemotherapy of Experimental *Staphylococcus aureus* Infections in Mice.

Preparation	Single dose, g/kg	Total dose, g/kg	Died before 20th day	Day on which death occurred (individual animals)	Pathology* on 20th day, all animals	Culture† from kidney 20th day, all animals
None (controls)			10/10	1,2,2,2,3,3,4,4,4,6	++++	++++
Sulfapyridine	2.0	34.0	8/10	4,4,5,5,6,8,9,10	+++ ++++	+++ ++++
Sulfanilyl sulfanilamide	2.0	34.0	4/10	1,2,8,15	0 + ++ ++ ++ ++++	§
Sulfathiazol	2.0	34.0	3/10	3,14,16	0 0 0 0 + ++ +++	0 0 0 0 + ++ +++
Sulfamethylthiazol	2.0	34.0	1/10	12‡	0 0 + + + + +++ +++ ++++	0 0 0 0 0 + + + +
Sulfaphenylthiazol	2.0	34.0	6/10	9,9,10,10,13,14	++ +++ +++ ++++	++++ +++ ++++ ++++

* See key at foot of Table I.
† Key: 0 Negative. + 4 to 6 colonies. ++ 10 to 100 colonies. +++ 100 to 200 colonies. ++++ 200 or more colonies.
‡ Mouse died of starvation; one small abscess on heart.
§ No culture tests.

Treatment with all compounds was carried out at the following time intervals: 1½, 7, 24 and 32 hours after inoculation and once each day thereafter until death occurred, or up to and including the 15th day. The preparations were administered as suspension in ¼ to ½ cc of milk (per single dose) by means of the stomach tube.

In the first experiment, all surviving mice were killed and necropsied on the 20th day. The results of this study, based on the pathological findings, are shown in Table I.

A second experiment was carried out in similar manner except that on the 20th day one kidney was removed and cultured. The results of this study, based on pathologic and bacteriologic findings, are shown in Table II.

At necropsy there was a striking contrast in the gross appearance of the tissues of mice treated with sulfathiazol and sulfamethylthiazol as compared with mice which had received sulfapyridine or minimal doses of sulfanilyl sulfanilamide. In a significant number of animals treated with these two thiazol compounds the kidneys, prostate, liver and spleen were essentially normal except for a few scars from healed abscesses. In several mice to which sulfathiazol and sulfamethylthiazol had been administered no staphylococcus infection could be demonstrated by pathological or bacteriological methods.

Conclusions. Sulfathiazol and sulfamethylthiazol prolong the life of mice infected experimentally with a highly mouse virulent strain of *Staphylococcus aureus* and prevent the development and allow healing of abscesses in kidneys and other organs in a significant number of animals.

11055 P

Effectiveness of Neoarsphenamine, Sulfanilamide, Sulfapyridine in Marrow Cultures with Staphylococci and Alpha Streptococci.

EDWIN E. OSGOOD. (With the technical assistance of Julia Joski.)

From the Division of Experimental Medicine, Department of Medicine, University of Oregon Medical School, Portland, Oregon.

Edward and John LeCocq[1] have reported beneficial effects of therapy with neoarsphenamine in patients with osteomyelitis and bacteriemia due to the hemolytic *Staphylococcus aureus.* Using the

[1] LeCocq, E., *West. J. Surg.*, 1936, **44**, 655; LeCocq, John, personal communication.

TABLE I.
Effect of Neoarsphenamine on Hemolytic *Staphylococcus aureus* Infections of Marrow Cultures.

Initial colonies per cc	Arsenic per cc in mg	Neoarsphenamine, mg per cc	No. of colonies per cc			
			4-6 hr	24 hr	48 hr	72 hr
Controls*	0.00	0.0	870	400,000,000		
5	2.06	6.4	0	0	0	
5	.01	.05	5	0	0	
10	.014	.045		0	0	
10	.012	.04		0	0	
10	.010	.03		0	0	
10	.07	.02		0	0	
11	.005	.15	5	0	0	
51	.004	.012	16	0	0	
11	.004	.012	0	0	0	
51	.003	.010	25	4	0	
3	.003	.009	0	0		
11	.003	.008	0	0	0	
13	.003	.008	14	16		
3	.003	.008	0	0		
3	.0025	.008	0	0		
13	.0025	.008	40	160	290	
51	.0025	.008	150	103		5
13	.002	.006	48	400	9,340	
51	.002	.006	40	5		2
3	.002	.006	0	0		
3	.0016	.005	0	0		
5	.0016	.005	15	184,000	199,000,000	
11	.0016	.005	25	25	12	
13	.0016	.005	192	49,000	250,000	
51	.0016	.005	40	25		1
3	.0014	.004	2	0		
13	.0012	.004	304	125,000	500,000	
3	.0011	.003	2	0		
10	.0002	.0006	15	282,000,000		
10	.0001	.0003	120	320,000,000		

* Controls were run for each experiment, but all were similar.

marrow culture technic,[2] the data given in Table I were obtained on hemolytic *Staphylococcus aureus* infections. Note that in all the controls the colony count reached 400,000,000 per cc in 24 hours. In those cultures containing over 15 parts per million of neoarsphenamine marrow cells were destroyed as well as the staphylococci. In those cultures containing over 9 parts per million of neoarsphenamine sterility uniformly resulted. In those cultures containing 3 to 6 parts per million of neoarsphenamine sterility frequently occurred, and in the others growth of the organism was greatly inhibited. Marrow cells were completely undamaged by 6 parts per million of the drug. In no culture did the presence of 1-10,000 concentrations of sulfanil-

2 Osgood, E. E., and Brownlee, Inez E., *J. A. M. A.*, 1937, **108**, 1793; Osgood, E. E., *J. A. M. A.*, 1938, **110**, 349; Osgood, E. E., *Arch. Int. Med.*, 1938, **62**, 181; Osgood, E. E., *J. Lab. and Clin. Med.*, 1939, **24**, 954.

amide or sulfapyridine result in sterility, and the colony counts were usually of the order of 12,000,000 per cc for sulfapyridine and 40,000,000 per cc for sulfanilamide at the time when the colony count in the control reached 100,000,000 per cc.[3]

Studies of alpha hemolytic streptococcus infections made by the same technic showed an even greater effectiveness of neoarsphenamine and an even lesser effectiveness of sulfanilamide and sulfapyridine.

Since the concentration of the drug must be above 3 parts per million for a period of 6 to 24 hours it would seem that frequent small doses or administration of neoarsphenamine by the method of continuous drip[4] should be more effective than single larger doses at longer intervals. Studies of the clinical effectiveness of this drug in staphylococcic bacteriemia and in subacute bacterial endocarditis and of the comparative effectiveness in marrow cultures of other arsenicals are in progress.

Summary. In cultures of living human marrow inoculated with hemolytic *Staphylococcus aureus* or alpha streptococci *(Streptococcus viridans)* neoarsphenamine in concentrations of 3 to 9 parts per million was far more effective than 1-10,000 concentrations of either sulfanilamide or sulfapyridine, and did not significantly damage marrow cells.

11056 P

Hyaluronic Acid in Pleura Fluid Associated with Malignant Tumor Involving Pleura and Peritoneum.

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Hyaluronic acid, a high molecular weight polysaccharide composed of acetylglucosamine and glucuronic acid in equimolar concentrations, has been obtained from the following sources: vitreous humor of cattle and swine,[1, 2] human umbilical cord,[1] cattle and human synovial

3 Osgood, E. E., Marrow Cultures, Figures 17-19 and 21, Symposium of the Blood and Blood-Forming Organs, University of Wisconsin Press, pp. 219-241, 1939.

4 Hyman, H. T., Chargin, L., Rice, J. L., and Leifer, *J. A. M. A.*, 1939, **113**, 1208.

1 Meyer, K., and Palmer, J. W., *J. Biol. Chem.*, 1936, **114**, 689.

2 Meyer, K., Smyth, E. M., and Gallardo, E., *Am. J. Ophthal.*, 1938, **21**, 1083.

fluid,[3] and group A hemolytic streptococci in the mucoid phase.[4] A polysaccharide acid closely resembling hyaluronic acid has further been obtained from tumors caused by a filterable fowl sarcoma.[5] An immunologically inactive polysaccharide acid resembling hyaluronic acid has likewise been demonstrated in group C hemolytic streptococci.[6] In the latter a close relationship between the yield of acid polysaccharide on one hand and the degree of encapsulation, virulence, and invasiveness on the other has been pointed out.

Many tumors are mucilaginous and many are stained by "mucin" stains. The nature of the "mucin" constituent, however, has been determined only in the case of the fowl sarcoma.

We have isolated hyaluronic acid from the viscous pleura fluid* of a patient with a malignant tumor of the pleura and peritoneum. Three different samples of pleura fluid were investigated. The procedure used for the isolation of the polysaccharides followed that described earlier.[3] The yield per 100 cc of fluid corresponded to 0.174, 0.187, and 0.142% as compared to 0.02-0.025% from bovine synovial fluid, and about 0.04% from bovine vitreous humor. The polysaccharides isolated from the pleural fluids had compositions and physical constants very similar to those reported previously. The identity was further established by enzymatic analysis.[7]

In previous papers from this laboratory it was pointed out that hyaluronic acid occurred free or in salt linkage only and was not chemically bound to protein.[1, 8, 9] In the present case the same conclusion could be drawn from electrophoretic measurements carried out by Dr. Longsworth.† At pH 7.8 in a 0.05 N lithium chloride-lithium diethylbarbiturate buffer and at 0°C a fraction with a mobility of -10.5×10^{-5} was observed, which was apparently the free polysaccharide acid. The concentration of this fraction as deter-

3 Meyer, K., Smyth, E., and Dawson, M. H., *J. Biol. Chem.*, 1939, **128**, 319.

4 Kendall, F. E., Heidelberger, M., and Dawson, M. H., *J. Biol. Chem.*, 1937, **118**, 61.

5 Kabat, E. A., *J. Biol. Chem.*, 1939, **130**, 143.

6 Seastone, C. V., *J. Exp. Med.*, 1939, **70**, 347, 361.

* We obtained the fluid and the clinical and pathological findings from Dr. R. Loeb to whom we are greatly indebted.

7 Meyer, K., Hobby, G. L., Chaffee, E., and Dawson, M. H., *J. Exp. Med.*, in press.

8 Meyer, K., Palmer, J. W., and Smyth, E. M., *J. Biol. Chem.*, 1937, **119**, 501.

9 Meyer, K., *Cold Spring Harbor Symposia on Quantitative Biology*, 1938, **6**, 91.

† We are greatly indebted to Drs. D. A. MacInnes and L. G. Longsworth of the Rockefeller Institute for Medical Research for the electrophoretic measurements and for their permission to quote the results in this paper.

mined from the electrophoretic measurements corresponded closely to the concentration of free polysaccharide as determined by isolation. Furthermore the mobility of the polysaccharides isolated from the tumor fluid as well as from umbilical cord were very similar to the mobility of the fast component in the tumor fluid.

From the clinical and pathological findings the present tumor was diagnosed as a meso- or endothelioma, a tumor apparently characterized in these locations by the mucilaginous nature of its effusates.[10] The following is part of the histological report from the Dept. of Pathology (Dr. E. Sproul):

"The sediment is a compact sheet of cells of uniform character. They are very large, rounded or polygonal mononuclear forms, usually with ovoid nucleus. The latter has a distinct membrane and delicate chromatin network. There are but rare mitotic figures. The cytoplasm is very abundant and vacuolated so that a delicate network of cytoplasm separates small vacuoles. The latter may be empty or contain slightly basophilic granular material with peripheral concentration of the homogeneous cytoplasm. The cells occur singly or in groups. There is no evidence of gland formation, but in one area the arrangement of the cells suggests a papillary structure. Despite the thick mucoid character of the fluid it has been impossible to stain mucin within cells or in the fluid by use of the mucicarmin stain. Many of the vacuoles seen in the tumor cells of the Zenker fixed material are shown to represent fat globules when formalin fixed material is stained with Scharlach R. The fat is very abundant and is present in large and small globules. That the presence of the visible fat does not represent an autolytic process is attested by the fact that the nucleus in the fat-laden cell is well preserved.

"Diagnosis: The large cells are those of a malignant tumor. It is felt that the uniformity of morphological features, the absence of mucin and glandular structures, the suggestion of papillary structure and the evidence of phagocytic activity strongly suggest that they are derived from the lining cells of the serosal cavity. The tumor would be classified as an endothelioma or mesothelioma of the peritoneum and pleura."

In regard to the failure of the mucicarmin stain of the tumor cells, one is reminded of a similar situation in the synovial cells which likewise produce hyaluronic acid. According to Kling[11] no granules indicating a specific function or secretion could be demonstrated with

10 Ewing, James, *Neoplastic Diseases*, Philadelphia and London, 1928, p. 350.

11 Kling, D. H., *The Synovial Membrane and the Synovial Fluid*, Los Angeles, 1938.

mucicarmin or mucihematin in the cells. Using a special technic of fixation the same author demonstrated metachromatic granules in synovial cells with Toluidin blue. Our own attempts to stain "mucin" in vitreous humor were all futile. Furthermore the capsule of group A hemolytic streptococci, which at least to a large extent is composed of hyaluronic acid, can only be stained by mucin stains with special technics.[12]

It is not known whether the normal endothelium produces hyaluronic acid in small amounts. However, the usual exudates and transudates of the serous cavities are not viscous.

Conclusion. Hyaluronic acid in high concentration has been isolated from the chest fluid of a patient with a malignant tumor, probably an endothelioma. The polysaccharide acid in the original fluid migrates in an electric field at pH 7.8 at essentially the same speed as the isolated pure polysaccharide acid, indicating the existence in the fluid of the free acid and not of a protein complex.

11057 P

Non-Osseous Origins of Serum Phosphatase.

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Associated increases of serum cholesterol, serum bilirubin and serum phosphatase have been observed in diseases of the liver with various degrees of obstruction of the biliary passages; *dissociated effects* have also been observed in other liver diseases.[1, 2] We have sought to reproduce analogous conditions and effects in experimental animals, in order to be able better to relate these dissociated effects to the underlying tissue changes.[3–6]

The effects of intravenous injections of gum acacia were of interest in this connection. We have obtained a marked *increase* of serum

[12] Hobby, G. L., and Dawson, M. H., *Brit. J. Exp. Path.*, 1937, **18**, 212.

[1] Bodansky, A., and Jaffe, H. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 107.

[2] Flood, C. A., Gutman, E. B., and Gutman, A. B., *Arch. Int. Med.*, 1937, **59**, 981; this paper reviews some earlier work.

[3] Bodansky, A., and Jaffe, H. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1179.

[4] Bodansky, A., *Proc. International Physiol. Cong.*, Leningrad, 1935, 253.

[5] Bodansky, A., *Enzymologia*, 1937, **3**, 258.

[6] Bodansky, A., *Proc. International Physiol. Cong.*, Geneva, 1938.

TABLE I.

Effects of a Single Intravenous Injection of 1.2 g of Gum Acacia per Kilo of Body Weight. Some figures are in italics to show the time of occurrence of the more significant effects.

Day	Hematocrit, %	Sedimentation rate mm/hr	Cholesterol, mg/100 cc				Proteins				Phosphatase units/100 cc
			Total	Esters	Free	Esters, % of total Cholest.	Albumin, %	Globulin, %	A/G ratio	Total, %	
					Dog 652.						
—8	55	0	139	98	41	70	4.0	2.5	1.6	6.5	1.5
—4	53	2	144	105	39	73	3.8	2.5	1.5	6.3	1.8
1	48	*38*	*100*	*69*	*31*	69	3.1	*1.9*	1.6	*5.0*	7.2
3	51	31	110	78	32	71	2.9	2.3	1.3	5.2	*9.0*
6	55	8	122	85	37	70	3.2	2.6	1.2	5.8	7.2
10	50	2	134	93	41	69	3.0	2.7	1.1	5.7	2.6
14	49	8	153	108	45	71	3.5	2.3	1.5	5.8	2.3
29	52	1	178	125	53	70	3.8	2.5	1.5	6.3	2.6
					Dog 811.						
—8	55	0	156	111	45	71	4.3	2.1	2.0	6.4	2.3
—4	54	0	148	105	43	71	4.1	2.2	1.9	6.3	2.2
1	51	*32*	*100*	*67*	*33*	67	3.3	*1.5*	2.2	*4.8*	7.2
3	50	8	128	93	34	72	3.4	1.8	1.9	5.2	*8.0*
6	52	0	105	69	36	66	3.9	1.8	2.2	5.7	6.8
10	49	1	120	86	34	72	3.3	*2.2*	1.5	5.5	3.1
14	50	1	141	102	39	72	4.1	1.6	2.6	5.7	3.3
29	51	0	129	90	39	70	4.2	1.8	2.3	6.0	3.2

phosphatase activity, with a striking decrease of cholesterol and of the ratio of cholesterol esters to the total cholesterol—a ratio which is generally considered one of the significant indices of liver function.

Experiment 1. Two dogs, weighing about 12 kilos each, were fasted for 20 hours before the intravenous injection of 1.2 g of gum acacia (in 8% solution) per kilo of body weight. Table I includes the more significant results of a month's observation; the time of occurrence of maximum effects is of interest:

Anorexia, retching and vomiting, one or more days after the injection must be mentioned, particularly in relation to the serum protein changes. The *hematocrit reading* decreased slightly, and the *sedimentation rate* increased markedly within 24 hours. *Serum albumin* reached within 24 hours a level near which it remained for 10 days; *serum globulin* reached its minimum on the 1st day and rose to a maximum on the 10th day; the *total protein* values represented a summation of these effects. *Total serum cholesterol* decreased to a minimum within 24 hours, largely at the expense of the ester fraction. *Serum phosphatase* rose to a maximum on the 3rd day.

Recovery to the initial values was substantially completed at various times before the end of the experiment, except for the serum phosphatase which remained significantly higher. In one dog the hematocrit reading remained slightly below the initial value and the cholesterol ester declined slightly after reaching the initial level; in the other dog, both cholesterol fractions rose above the initial levels.

Experiment 2. Examination of Table I indicated immediate effects much greater than those observed after 24 hours. The experiment was repeated, with the same dogs. The results are given in Table II. The values observed after 2 and 6 hours were of particular interest.

The total serum proteins and the globulin fraction reached their minimum values within 2 hours; serum albumin decreased relatively less, and the subsequent changes were less definite than those of the globulin fraction. *Cholesterol esters* declined drastically within 2 to 6 hours; free cholesterol decreased in one dog and remained substantially constant in the other. Serum phosphatase rose about 50% within 2 hours.

Summary and Conclusions. Serum globulin participated relatively more in the changes of serum protein content, causing at first a decrease and then an increase of the albumin/globulin ratio before the final return to initial values. Cholesterol esters decreased absolutely and relatively more than the free cholesterol fraction. Serum phosphatase activity rose promptly, continued to rise for 3 days and

TABLE II.
Effects of a Second Intravenous Injection of Gum Acacia Solution, One Month After the First.

Hour	Hematocrit, %	Sedimentation rate mm/hr	Cholesterol, mg/100 cc: Total	Esters	Free	Esters, % of total Cholest.	Proteins: Albumin, %	Globulin, %	A/G ratio	Total, %	Phosphatase units/100 cc
					Dog 652.						
0	52	1	178	125	53	70	3.8	2.5	1.5	6.3	2.6
2	43	36	127	90	37	71	3.0	*1.6*	1.9	*4.6*	4.0
6	44	41	*81*	*45*	36	*56*	2.9	1.9	1.5	4.8	4.9
24	45	32	126	81	45	64	3.1	1.8	1.7	4.9	*8.2*
					Dog 811.						
0	51	0	129	90	39	70	4.2	1.8	2.3	6.0	3.2
2	48	3	*68*	*34*	*34*	50	3.7	*1.4*	2.6	*5.1*	4.4
6	47	10	99	57	42	58	3.8	1.5	2.5	5.3	5.3
24	50	0	124	83	41	67	3.9	1.7	2.3	5.6	*6.2*
48	54	0	134	97	37	73	4.0	1.6	2.5	5.6	6.1

was still above the initial levels a month after the injection of gum acacia; we consider this a manifestation of the reaction of the liver to injury. In absence of criteria which would enable us to identify in the serum the "alkaline" phosphatases of various origins, we regard the rise of serum phosphatase in liver involvements—particularly when there is no evidence of obstruction of the biliary passages—as an indication that the liver is a source of serum phosphatase. We assume that the "alkaline" phosphatase in the serum represents the sum total of contributions from various organs and tissues capable of producing "alkaline" phosphatase, the contribution of each being increased by an injury to which it is able to react.

This study, including histological investigations, is being continued.

11058

Cure and Prevention of Vitamin E-Deficient Muscular Dystrophy with Synthetic α-Tocopherol Acetate.

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It is now well established that adult rats reared from birth or weaning on a vitamin E-deficient diet eventually develop a muscular dystrophy.[1-3] It has also been shown that muscle changes, characteristic of dystrophy, can be detected before overt symptoms appear.[4] The criteria offered were (1) increase in the concentration of water and (2) of chlorides of the muscle, (3) decrease in the maximum strength, (4) focal hyaline necroses of muscle fibers, and (5) decrease in the creatine concentration of the muscle. Using the first 4 criteria it has been shown that wheat germ oil would prevent the development of the dystrophy.[4] It was later shown that wheat germ oil or its vitamin E-active concentrate would induce recovery from

1 Blumberg, Harold, *J. Biol. Chem.*, 1935, **108**, 227.

2 Burr, G. O., Brown, W. R., and Moseley, R. L., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 780.

3 Ringsted, A., *Biochem. J.*, 1935, **29**, 788.

4 Knowlton, G. C., and Hines, H. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 655.

all the symptoms, except the loss of muscle strength, when fed to animals with an established dystrophy.[5]

The above experiments do not eliminate the possibility that the effect of wheat germ oil may be due, not to vitamin E itself, but to some other component of the wheat germ oil. The present experiments show, however, that synthetic vitamin E (d-l α-tocopherol acetate, *i. e.*, Ephynal*) is effective in the prevention and cure of the muscular dystrophy.

Methods. Female rats, when obviously pregnant (16-18th day of gestation), were placed on a vitamin E-deficient diet[6] and maintained on this diet throughout lactation. Males only were used, and these were weaned at 24 days of age and continued on the vitamin E-deficient diet throughout the experiment. The development and course of this dystrophy were studied in rats at ages of 1, 2, 3, 4, 5 and 8 months.

The curative effects of α-tocopherol were tested on a group of E-deficient dystrophic male rats which, beginning at an age of 5 months, were given weekly subcutaneous injections of 3 mg of α-tocopherol acetate in 0.1 cc olive oil. These weekly supplements were continued until the animals reached an age of 8 months, at which time the muscles were studied. A group of litter mate controls were given weekly subcutaneous injections of 0.1 cc of olive oil over the same period of time.

In the experiments testing the preventive effects of α-tocopherol, the weekly supplements were given in the same amount and manner as described above. The supplements were started at 30 days of age, at which time the characteristic dystrophic changes had not yet appeared, and were continued until 150 days of age, when the muscles were studied for evidences of dystrophy.

The gastrocnemii of each animal were studied as to (1) maximum strength, (2) water concentration, (3) chloride concentration, and (4) histologic appearance. The methods used have been described previously.[5]

Results. Muscle strength at 1 month of age is normally about 55% of the adult value. Water and chloride concentrations are higher in young animals than in the adult.[7] In the present paper we

[5] Knowlton, G. C., Hines, H. M., and Brinkhous, K. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 453.

* Ephynal was furnished through the courtesy of Dr. E. D. Shaner of the Hoffmann-LaRoche Laboratories, Inc.

[6] Olcott, H. S., *J. Nutrition*, 1938, **15**, 221.

[7] Hines, H. M., and Knowlton, G. C., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 133.

TABLE I.
Development of Muscular Dystrophy on a Vitamin E-Deficient Diet.

Age in months	No. of animals	Muscle			
		Max. strength, % of normal	Water, % of normal	Chloride, % of normal	Necrotizing lesions
1	3	59.	98.9	98.	0
2	4	73.	100.1	103.	+++
3	4	74.	100.9	112.	++
4	4	86.	100.8	115.	++
5	6	69.	100.4	117.	++
8	5	70.	101.1	147.	++

have compared deficient animals with normal animals of the same age and strain, and the values given in the tables are expressed in percentage of the normal levels at that same age. In addition to these data, the tables also show the approximate extent of the focal necrotizing lesions in each group. In most animals, even with well-developed dystrophic changes, only a few fibers in any one section showed degenerative changes. Associated with the hyaline necrosis

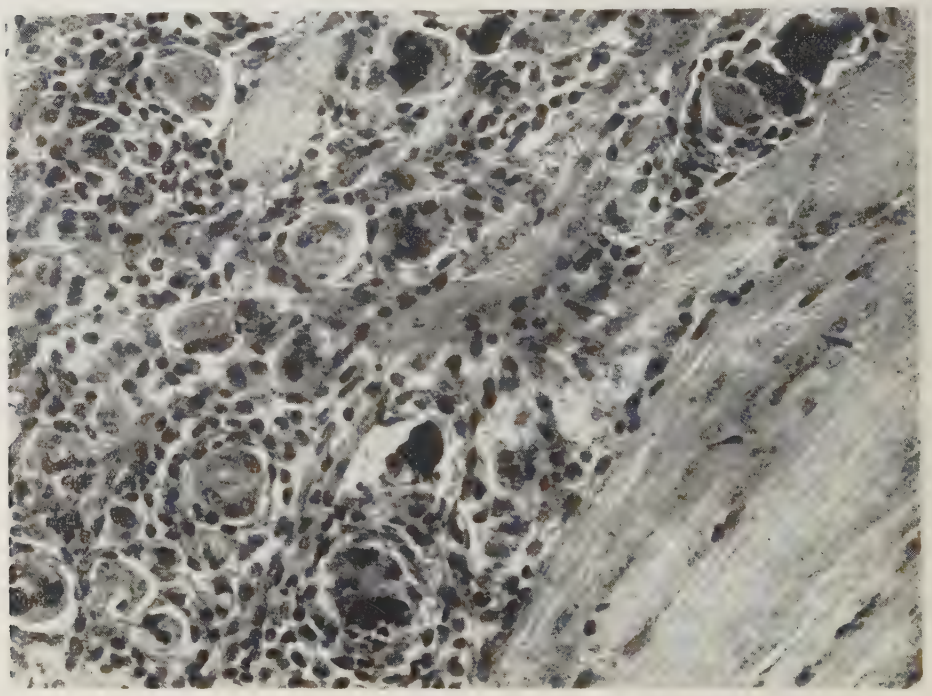

FIG. 1.
Gastrocnemius muscle from a vitamin E-deficient rat, 2 months of age. Marked proliferation of myocytes. Partial calcification of necrotic muscle substance. × 350.

there were often reactive inflammatory changes and beginning regeneration of muscle fibers. In addition to these lesions, many intact fibers in the dystrophic muscles showed an increase in the number of subsarcolemma nuclei (see also Evans, Emerson, and Telford,[8] Knowlton, Hines, and Brinkhous[5]). These proliferative changes are interpreted as a late stage of regeneration or as a reaction to a mild injury not sufficient to cause necrosis.

From Table I it is seen that dystrophic changes are clearly evident by the time the deficient animals reach the age of 2 months. At the age of one month there is some evidence of muscle weakness. However, the number of animals used was small and the individual variations are so great in animals of this age that the value obtained is not significantly different from normal. The other criteria of dystrophy were entirely absent at this age.

Beginning with the second month the muscle strength improved somewhat up to the fourth month of age, but thereafter it suffered

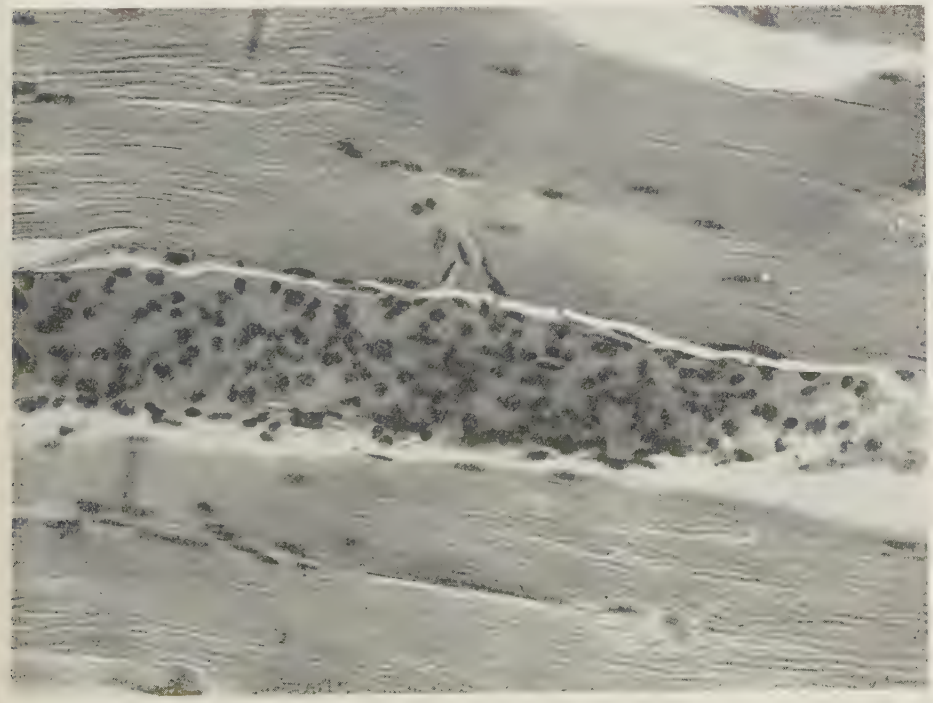

FIG. 2.

Gastrocnemius muscle from a vitamin E-deficient rat, 8 months of age. Isolated necrotic muscle fibre. × 350.

8 Evans, H. M., Emerson, G. A., and Telford, I. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 625.

considerable impairment. The concentrations of water and chlorides increased steadily from the very beginning.

The histologic lesions, although very patchy in distribution, were most severe at the end of the second month. One muscle at this age which showed very marked changes histologically (Fig. 1) also showed gross lesions. After the second month the necrotizing lesions were much less extensive (Fig. 2). Proliferative muscle lesions were observed in all deficient animals after the first month of age.

TABLE II.
Cure of Nutritional Muscular Dystrophy with α-Tocopherol (Rats 8 Months of Age).

		Muscle			
No. of animals	Supplement	Max. strength, % of normal	Water, % of normal	Chloride, % of normal	Necrotizing lesions
6	α-tocopherol	96.	99.6	111.	0
5	olive oil	73.	100.8	150.	++
5	none	70.	101.1	147.	++

FIG. 3.
Gastrocnemius muscle from an 8-months-old rat on a vitamin E-deficient diet. Treated with α-tocopherol acetate since age of 5 months. Practically complete recovery. × 350.

TABLE III.
Prevention of Nutritional Muscular Dystrophy by Use of α-tocopherol (Rats 5 Months of Age).

No. of animals	Supplement	Muscle			
		Max. strength, % of normal	Water, % of normal	Chloride, % of normal	Necrotizing lesions
12	α-tocopherol	78.	99.4	95.	0
11	olive oil	66.	100.4	120.	++
6	none	69.	100.4	117.	++

α-tocopherol acetate caused complete recovery of an established dystrophy in the deficient rats (Table II). In those dystrophic rats which were started on the supplement at 5 months of age and continued on this regimen to 8 months of age, the characteristic dystrophic changes were absent. The maximal strength and water and chloride concentrations had returned to a normal or nearly normal level, and necrotizing muscle lesions were absent. The proliferative muscle lesions, not recorded in the table, had practically disappeared. Only a very few muscle fibers showed increase in the number of subsarcolemma nuclei. Fig. 3 shows an area of muscle which is fairly representative of this group of rats cured of the dystrophy.

Table III shows the effect of α-tocopherol in preventing lesions in animals maintained on the deficient diet. Injections of α-tocopherol were given weekly, beginning at one month of age. At the end of 5 months, the muscle strength was somewhat subnormal, but was definitely greater than in the control groups. The water and chloride concentrations were normal and necrotizing lesions were entirely absent. Proliferative muscle lesions did develop, however.

From these data, it appears that α-tocopherol acetate in the dosage used, is even more effective in promoting a cure in the older animals than in preventing symptoms in the younger animals. This may depend upon: (1) a greater requirement during the period of rapid growth, and (2) a less efficient utilization of the injected α-tocopherol ester by the younger animals.

Conclusion. From these results it may be concluded that subcutaneous injections of synthetic α-tocopherol acetate are effective in both the cure of and the prevention of the nutritional muscular dystrophy seen in rats maintained from an early age on a vitamin E-deficient diet.

11059

Thyroid Treatment and the Cyclostome Endostyle.

MIRIAM STOKES. (Introduced by J. Frank Daniel.)
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A relationship between the thyroid gland and metamorphosis has been fully established in amphibians,[1] and has been suggested for at least one species of fish.[2] Two factors appear to be necessary for that relationship, an actively secreting gland, and sensitivity of the body cells to its hormone.

The adult cyclostome possesses a single thyroid gland which develops at metamorphosis from the endostyle, a mucus-secreting structure ventral to the pharynx. According to Horton,[3] the thyroid of the adult lamprey will induce metamorphosis in tadpoles. Subjection of larval lampreys to thyroid treatment has produced no effect on external metamorphosis;[4] and potassium iodide solutions, effective when administered to amphibian larvae, do not alter the histology of the endostyle.[5]

The present study was undertaken to test the results of earlier investigators with a larger number of animals. A careful histological study of the endostyle was also made, on the possibility that slight metamorphic changes not reflected in external appearance might have been produced by thyroid treatment.

Ammocoetes larvae 50-80 mm in length, of the genus Entosphenus, were collected in midsummer and divided into lots of 20. Larger animals were discarded to eliminate the complication of normal metamorphosis, and the larvae were placed in finger bowls in groups of 5. The solutions used were as follows:

1. Control—pond water.
2. Crystalline thyroxin* in pond water, 1:500,000.
3. Desiccated thyroid (crushed tablets), 1:120,000.
4. Desiccated thyroid (powder), 1:500,000.

(A similar concentration of this preparation produced dis-

1 Gudernatsch, J. F., *Arch. f. Entw.-mech.*, 1912, **35**, 457, and others.

2 Murr, E., and Sklower, A., *Z. f. vergl. Physiol.*, 1928, **7**, 279.

3 Horton, F. M., *J. Exp. Biol.*, 1934, **11**, 257.

4 Jensen, C. O., *Oversigt. over det kgl. danske Videnskabernes selskabs Forhandlinger*, 1916, **30**, 251; Remy, P., *Compt. rend. Soc. biol.*, 1922, **86**, 129.

5 Marine, D., *J. Exp. Med.*, 1913, **17**, 379.

* The thyroxin used was furnished through the courtesy of E. R. Squibb and Sons.

tinct metamorphic changes in Hyla tadpoles in 7 days. Stronger doses were fatal.)

In contrast to most of the earlier work, where animals were exposed only at intervals, the Ammocoetes were kept in the solutions, which were renewed every 3 days.

In all groups the results were consistently negative. No external evidence of metamorphosis was present, and no effect on size was noted. Histological examination of structures most influenced by metamorphosis, the eyes, the oesophagus, and particularly the endostyle, showed no changes. Experimental animals were indistinguishable from controls in section. Thus, although the metamorphosis of the lamprey endostyle is normally completed in a month, thyroid substance produced no effect on its cellular structure during a 6-week exposure.

From the data collected it may be postulated that although the adult lamprey possesses an active thyroid, the body cells of Ammocoetes, including the cells of the endostyle, are not sensitive to thyroid treatment. Metamorphosis in cyclostomes, therefore, is related to influences other than that of the developing thyroid.

11060

Action of Mammalian Sex Hormones in the Lizard, *Sceloporus occidentalis*.

AUBREY GORBMAN. (Introduced by J. Frank Daniel.)

From the Department of Zoology, University of California, Berkeley.

With the discovery of an increasing number of estrogens and androgens in mammals, and an increasingly diverse series of physiological activities in which they are involved, it has become of great interest to examine the action of such substances in lower vertebrates. In adult frogs, injection of estrogens prevents atrophy of the oviduct after ovariotomy,[1] and high doses (1,000 rat units) stimulate growth of the oviduct in normal animals.[2] In normal toads, however, dosages of estrone as high as 10,000 international units have little or no demonstrable action.[3, 4] A surprising result is ovulation in

1 Wolf, O. M., *Anat. Rec.*, 1928, **29**, 41.

2 Wolf, O. M., *Biol. Bull.*, 1939, **77**, 338.

3 Valenzuela, J. R., *Rév. Méd. Cordoba*, 1937, **25**, 179.

4 de Allende, I. C., *Rev. Soc. Arg. Biol.*, 1939, **15**, 185.

Xenopus laevis in response to progesterone, testosterone, and allied compounds, but not to estrogenic substances, such as estrone, estriol, and estradiol.[5, 6] In young alligators estrone stimulates the ovary and oviduct; testosterone also stimulates the oviduct.[7, 8] In lizards testosterone induces secretory activity in "sexual segments" of urinary tubules, and an increase in the size of the oviduct.[9] Estrone, however, produces a marked reduction in testicular size in lizards, but stimulates the epididymis and Wolffian duct.[10, 11]

In this report the results of administration of theelin and testosterone propionate (Oreton)* to adult Fence Lizards *Sceloporus occidentalis,* of both sexes are outlined. Dosages of each hormone used were either .4 cc (.08 mg theelin, 4.0 mg testosterone) given over a period of 5 days, or .5 cc (.10 mg theelin, 5.0 mg testosterone) given over a period of 2 weeks. The difference in effect between the two modes of treatment was only slight, a more pronounced effect being found in each case in the .5 cc group. Twenty-one animals of uniform size and weight were examined. Seven lizards (4♀, 3♂) which were not injected were used as controls. Seven lizards (4♀, 3♂) received testosterone, and 7 (4♀, 3♂) were given theelin. At autopsy the entire urogenital system, the adrenals, and the thyroid were fixed and serially sectioned.

No significant effects of hormone treatment were found in the thyroid, adrenal, ovary, or mesonephros.

Testes were not affected by testosterone, but were slightly reduced in size by theelin. In one exceptional animal reduction in testicular size was so extreme as to throw the tunica albuginea into deep folds.

Epididymides were reduced 20% in diameter by theelin in males. Tubular epithelial cells were reduced to 6μ in height, in contrast to 11μ in control males. Testosterone, on the other hand, slightly stimulated the female epididymis, and markedly enlarged the male epididymis, doubling its diameter. Tubules of such enlarged epididymides were increased 25% in width, and filled with secretion "colloid". The epithelial cells became very high columnar (35μ).

Mitotic figures in the Wolffian duct of the male were increased in

5 Shapiro, H. A., *J. Soc. Chem. Ind.*, 1936, **55**b, 1031.

6 Zwarenstein, H., *Nature*, 1937, **139**, 112.

7 Forbes, T. R., *Anat. Rec.*, 1938, **72**, 87.

8 Forbes, T. R., *Science*, 1938, **87**, 282.

9 Kehl, R., *Compt. rend. Soc. biol.*, 1938, **127**, 142.

10 Turner, C. D., *Biol. Bull.*, 1935, **69**, 143.

11 Clapp, M. L., *Anat. Rec.*, 1937, **70**, suppl. No. 1, 97.

* Theelin was furnished by Parke, Davis & Co. through the courtesy of Dr. Oliver Kamm, and Oreton by Schering Corporation through the courtesy of Dr. Max Gilbert.

number by theelin to 8.5 per cross section, and cell height was raised from high cuboidal (in control males) to high columnar. No effect of theelin was distinguishable in the Wolffian duct of the female. In neither sex did theelin increase the diameter of the duct. Testosterone did enlarge the width of the female Wolffian duct slightly, and mitoses were increased to 17.1 per cross section. Testosterone produced similarly slight enlargement in the Wolffian duct of the male, but mitoses were only 11.0 per cross section.

The most marked effects of theelin and testosterone injection were seen in the oviduct. Theelin produced an increase of 40% in cross-sectional diameter, and of more than 100% in thickness of the wall. This increase was due to a proportional thickening of the mucosa and the mucous glands situated beneath the mucosa. Testosterone elicited an increase of 57% in the diameter of the oviduct, and 115% in the thickness of its wall. However, the increase in thickness of the wall was due almost entirely to enlargement of mucous glands, the mucosa appearing stretched over the increased surface. Evidence of secretory activity in theelin- and testosterone-treated oviducts, but not in controls, appeared in the form of a large amount of methyl green-staining (mucous?) material in the lumen. No increase in mitotic activity was apparent in any of the treated oviducts.

11061

Effects of Testosterone Propionate on the Female Viviparous Teleost, *Xiphophorus helleri* Heckel.

Francis Marsh Baldwin and Howard Sidney Goldin.

From the Physiological Laboratories, University of Southern California, Los Angeles, California.

Experimental sex-reversal and the study of intersexes in the vertebrates have been of fundamental importance in the analysis of the rôle of the genes and endocrine secretions in embryonic development, particularly in sex-differentiation.

Gallagher, *et al.*,[1] indicated that urine from adult men and women contains both sex hormones and that the ratio of androgen to estrogen is higher in the urine of males than it is in the urine of females. The results obtained by Callow[2] substantiated the work of Gallagher,

1 Gallagher, *et al.*, *J. Clin. Invest.*, 1937, **16**, 695.

2 Callow, *Proc. Royal Society*, 1938, **31**, 841.

et al. Dingemanse, *et al.*,[3] reported findings that are not altogether harmonious with the work just cited but stated that the androgen-estrogen ratio was similar in the urines of the two sexes. This evidence indicates that each sex is hermaphroditic regarding the sex hormones, the difference being a quantitative one.

Since these differences of the sex hormones' content of the respective urines of the sexes are quantitative and not qualitative, and since they seem proportionately not to be very great, the study of the effect of the synthetically prepared male sex hormone, testosterone propionate,* upon the viviparous teleost, *Xiphophorus helleri* Heckel, was initiated to determine if, and to what extent, the relatively greater amount of one sex hormone checks the development and activity of the functioning sex tissue and accessory ducts of the other sex, and stimulates its own sex ducts and tissue to develop, and to learn if such development be caused by the establishment of a relatively greater amount of the male sex hormone in the female.

Essenberg[4] theorized, from histological examinations of 2 cases of natural complete sex-reversal observed in several hundred fish, that about 50% of all females *might* undergo sex-inversion, but did not necessarily *have* to do so. He concluded that any agent or condition which tends to decrease the capacity for female sex hormone secretion beyond a certain limit, became an immediate factor in the possibility of the sex-reversal in the female of the *X. helleri.*

Witschi and Crown[5] added testosterone to the water in the aquaria in which they kept pregnant *X. helleri* and obtained abortion and resorption of the young. In non-pregnant adult females, under the above conditions, all the large eggs underwent resorption. All treated females developed ovaries that resembled testes, but spermatogenesis was not reported, although the treated fish assumed gradually but completely the male secondary sex characteristics. Regnier,[6] using intra-muscular administration of the male sex hormone, reported that the sexual development of approximately one-third of her treated young female *X. helleri* developed male secondary sex characteristics.

This report involved experiments upon 91 virgin females and 7 normal males of the species. All of the subjects were past the un-

[3] Dingemanse *et al., Biochem. J.*, 1937, **31**, 500.

* By the courtesies of Dr. G. Stragnell of the Schering Corporation, and of the Ciba Pharmaceutical Products, Inc.

[4] Essenberg, J. M., *Biol. Bull.*, 1926, **51**, 98.

[5] Witschi, E., and Crown, E. N., *Anat. Rec.*, 1937, **70**, 121.

[6] Regnier, M. T., *Bull. Biol. de la France et de la Belgique*, **79**, 385 pp.

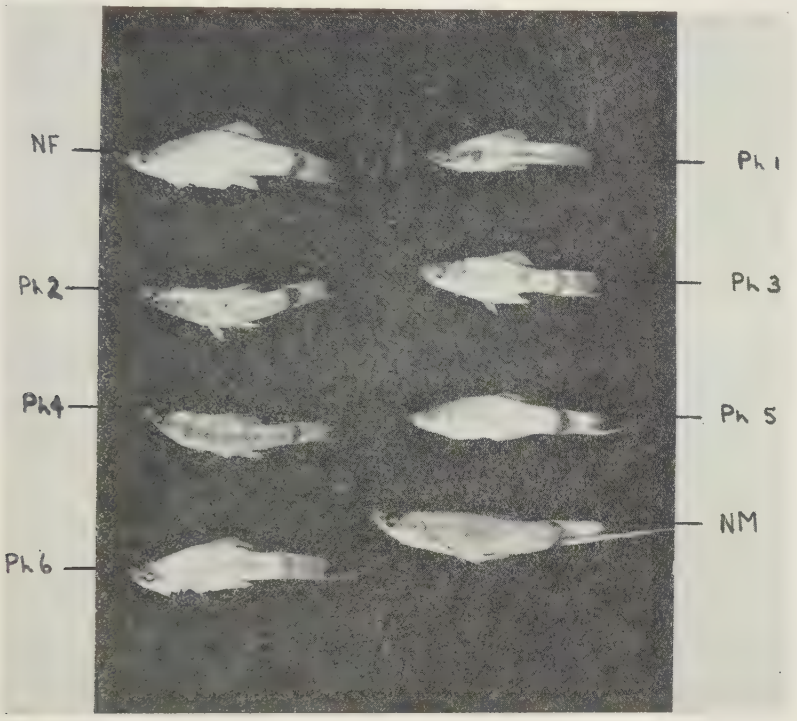

PLATE 1.

Progressive Phases of Development of the Artificially Induced Male Secondary Sex Characteristics in the Treated Females.

NF	Normal Female	NM	Normal Male
Ph 1	Phase 1	Ph 2	Phase 2
Ph 3	Phase 3	Ph 4	Phase 4
Ph 5	Phase 5	Ph 6	Phase 6

differentiated stage, with their sex being definitely established.

The testosterone propionate† was injected retroperitoneally, approximately 2 mm anterior to the anus, ventrally and laterally. Each fish received 0.5 mg testosterone propionate in 0.02 cc sesame oil weekly. The experiments extended over a period of approximately 19 weeks, with the progressive changes shown in Plate 1. The majority of treated fish reached phase 1 at an average time of 10.5 days after the initial injection; subsequent phases were reached on the averages of 21.9 days, 31.1 days, 45.4 days, 57.8 days, and 69.1 days after the initial injections, respectively.

† Withdrawn and used immediately from the original ampules furnished through the courtesies of the Schering Corporation and the Ciba Pharmaceutical Products, Inc.

Controls of several types were used. First, one-sixth of the total number of virgin females were left absolutely untouched. Second, a similar number of virgin female fish were treated only with pure sesame oil Third, normal males of the species were used as the basis of comparison of the extent of morphological and histological transformations expressed in the degree of apparent sex-reversal of the experimentally treated fish.

Not only does the experimental administration of testosterone propionate cause the regular morphological changes (Plate 1) in all

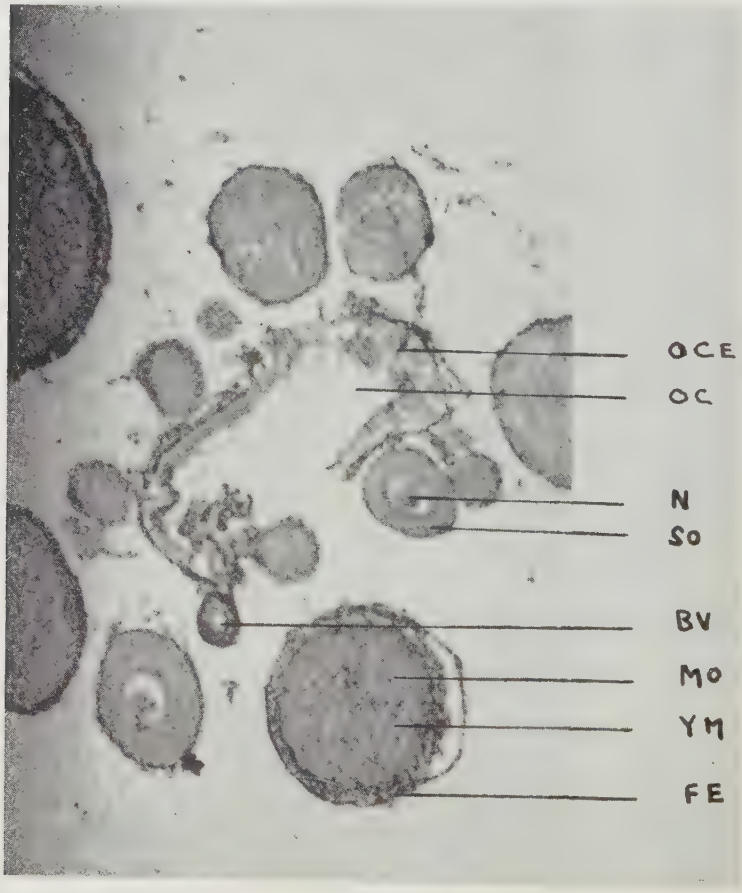

Plate 2.

Cross-section of treated female gonad: resorption. BV, Blood Vessel. FE, Follicular Epithelium. MO, Medium-sized Oöcyte. N, Nucleus. OC, Ovarian Cavity. OCE, Ovarian Cavity Epithelium. SO, Small-sized Oöcyte. YM, Yolk Material.

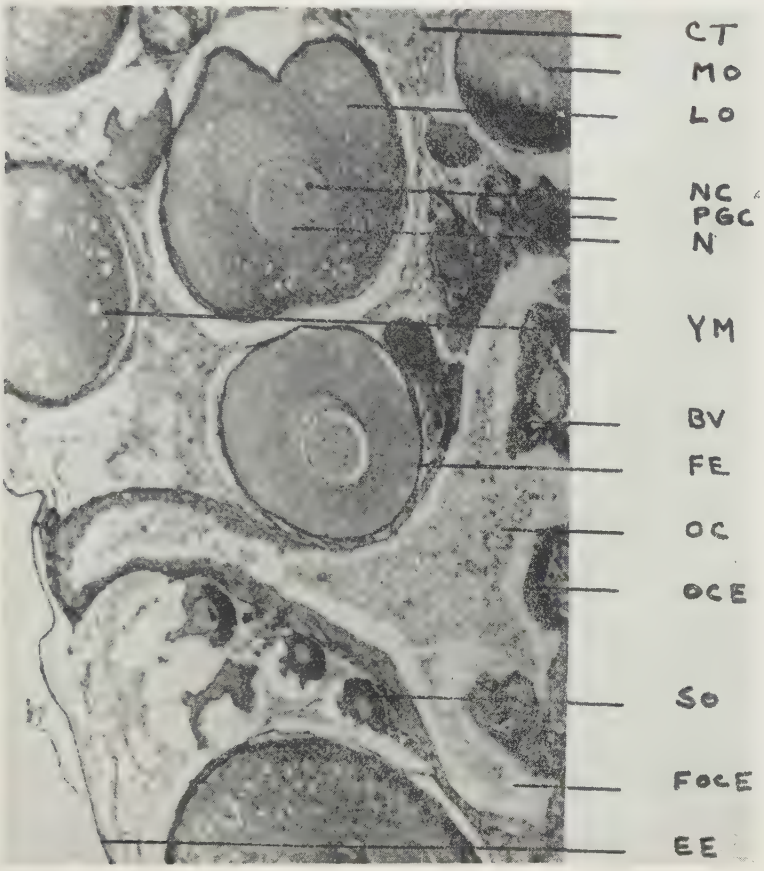

Plate 3.

Cross-section of normal female gonad. BV, Blood Vessel. CT, Connective Tissue. EE, External Epithelium. FE, Follicular Epithelium. FOCE Folds of Ovarian Cavity Epithelium. LO, Large-sized Oöcyte. MO, Medium-sized Oöcyte. N, Nucleus. NC, Nucleolus. OC, Ovarian Cavity. OCE, Ovarian Cavity Epithelium. PGC, Primordial Germ Cells. SO, Small Oöcyte. YM, Yolk Material.

cases to simulate the male in body color, formation of a caudal sword, and the development of the anal fin into the copulatory organ, the male gonopod, but these changes are accompanied by histological changes in the primary sex organs in approximately 50% of the experimentally treated virgin female fish. Females which respond to such treatment in these experiments are characterized as showing either (a) resorption of the gonad (Plate 2), or (b) some phase of spermatogenesis. Individuals which are completely altered histologically apparently pass from normal female gonadal structure

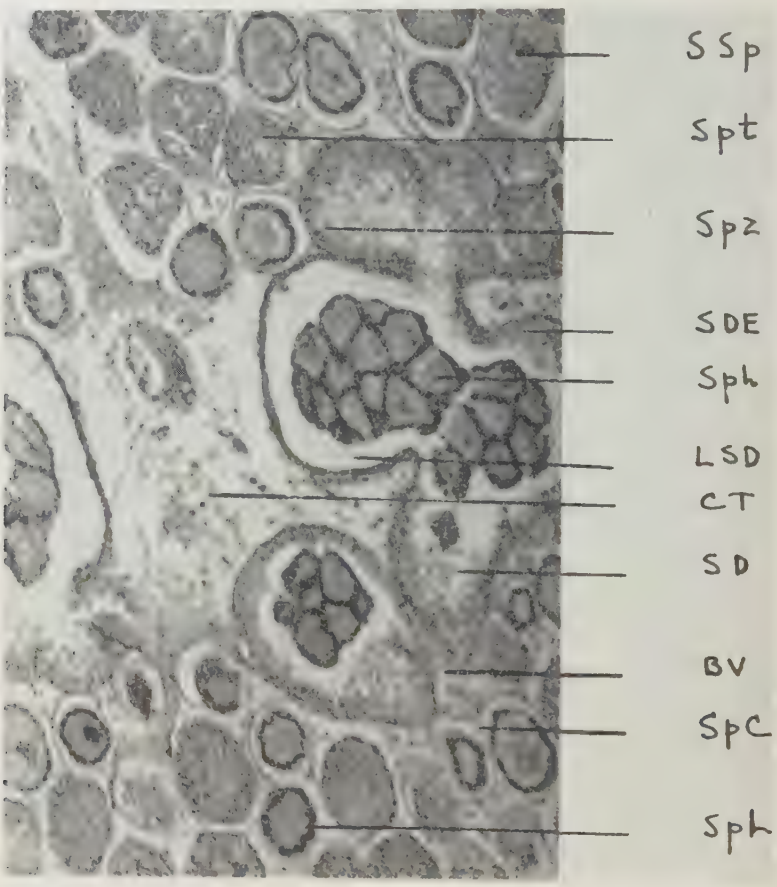

Plate 4.

Cross-section of treated female gonad: Spermatogenesis, Phase 6. BV, Blood Vessel. CT, Connective Tissue. LSD, Lumen of Sperm Duct. SD, Sperm Duct. SDE, Sperm Duct Epithelium. SpC, Spermatocyst. Sph, Spermatophores. Spt, Spermatids. Spz, Spermatozoa. SSp, Secondary Spermatocytes.

(Plate 3) through progressive resorption stages and finally show histological features (Plate 4) resembling those of the normal male gonad (Plate 5). These results substantiate Essenberg's[4] postulation, previously mentioned. It is thus to be concluded that the administration of the esterified androgen, testosterone propionate, to virgin female *Xiphophorus helleri* Heckel, causes sex-reversal in approximately 50% of the experimentally treated animals. However, it is not to be concluded that the appearance of changes in secondary sex characteristics are to be taken as a certain index of complete sex-reversal in this form.

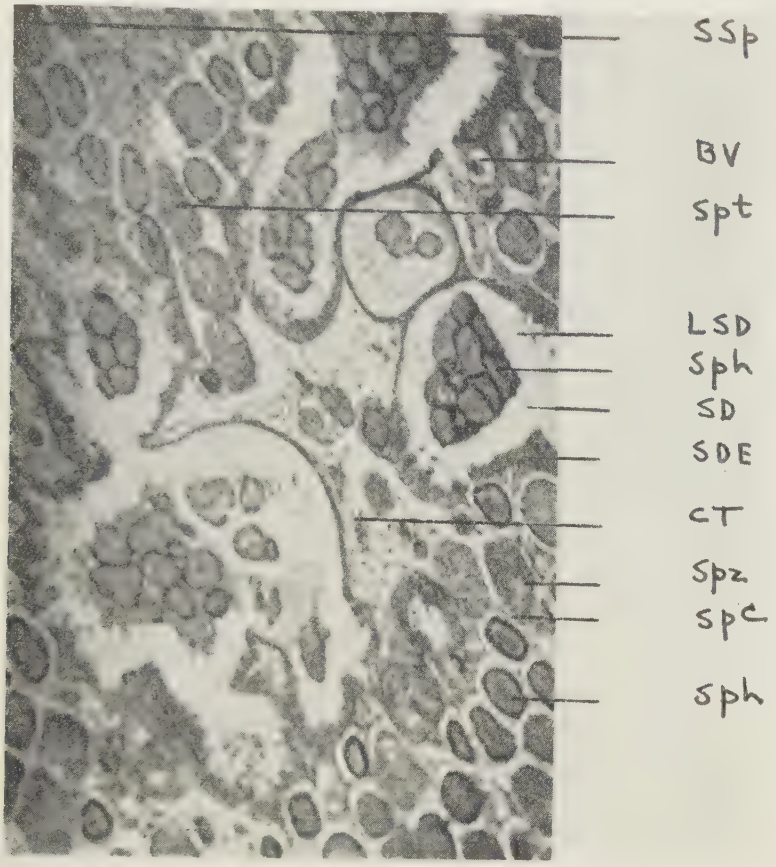

PLATE 5.

Cross-section of Control Male Gonad. BV, Blood Vessel. CT, Connective Tissue. LSD, Lumen of Sperm Duct. SD, Sperm Duct. SDE, Sperm Duct Epithelium. SpC, Spermatocyst. Sph, Spermatophores. Spt, Spermatids. Spz, Spermatozoa. SSp, Secondary Spermatocytes.

11062

Masculinization of Adult Female Rabbit Following Injection of Testosterone Propionate.

C. K. Hu and C. N. Frazier.

From the Division of Dermatology and Syphilology, Department of Medicine, Peiping Union Medical College, Peiping, China.

Lipschütz,[1] working in Steinach's laboratory, observed that the transplantation of the testis into spayed guinea pigs produced growth of the corpora cavernosa of the clitoris, and transformed this structure into a penis-like organ. Since then, as pointed out by Moore,[2] some of the many effects of the various chemical androgens indicate that any masculine rudiments, or homologues of male structures that remain during sexual differentiation, may develop typical masculine responses.

Although the effect of testosterone propionate on the female genital organs has been studied by a number of workers,[3, 4] chiefly in immature rats and mice, no observation seems to have been made either on the immature or adult rabbit. Moore and Price[5] found that the the injection of androsterone into young rats reduced testicular weights, whereas in mature animals, the testes were not injured. This last observation might be taken as indicative of a difference in the effect of the substance on the sexual organs of immature and mature animals.

To our knowledge only de Jongh and Mulder[6] have studied the masculinizing effects of "male hormone" on the adult animal, in this case, the guinea pig. Furthermore, the influence of testosterone on psycho-sexual behavior, except in birds,[7] has apparently not attracted the attention of previous workers. It may be of interest, therefore, to record the observations we have made on adult female rabbits treated with this substance.

Material and Method. To each of 3 adult female rabbits between

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1 Lipschütz, A., *Arch. f. Entwickl.-Mech.*, 1918, **44**, 196.

2 Moore, C. R., *Sex and Internal Secretions*, edited by E. Allen, 2nd edition, Williams & Wilkins, Baltimore, 1939, **7**, 420.

3 Lacassagne, A., and Raynaud, A., *Compt. rend. Soc. de biol.*, 1937, **125**, 351.

4 Greene, R. R., Burrill, M. W., and Ivy, A. C., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 4.

5 Moore, C. R., and Price, D., *Endocrinol.*, 1937, **21**, 313.

6 de Jongh, S. E., and Mulder, J. D., *Endokrinol.*, 1932, **11**, 161.

7 Shoemaker, H. H., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 299.

8½ and 15½ months of age, testosterone propionate* was injected subcutaneously in the dosage of 1 mg per day, 6 days a week. The treatment lasted from 21 to 32 days. Examination of the animals for the physiological effects of the preparation was carried out at intervals of 3-7 days. Attention was paid especially to anatomical changes in the external genitalia, the nipples, and the dewlap, a secondary sexual character of the female rabbit.[8] The behavior of the masculinized animals when confronted with normal female and male rabbits was also studied. One of the 3 animals was ovariectomized 6 months after the last injection. Both ovaries together with the surrounding fat and part of the fallopian tubes were removed under ether anesthesia. At the time of operation, all the changes induced by androgenic treatment had disappeared, the animal having returned to an apparently normal female status. Beginning a week after operation, testosterone was again administered.

Effect on Somatic Sexual Characters. The response to treatment was strikingly uniform in the 3 animals. Change in appearance of the clitoris and labia was noticeable within 3 or 4 days after the beginning of treatment. Between 10 and 14 days after the first injection of testosterone, the clitoris enlarged, and the labia became red and swollen, and then began to fuse posteriorly. The process of hypertrophy continued until the phallus resembled a typical penis in so far as the corpora cavernosa of the clitoris were concerned. When viewed from the anterior surface the glans-like structure could not be distinguished from the penis of a normal rabbit. A prepuce-like structure also developed which could be retracted with better exposure of the newly formed glans. The vaginal opening, although reduced to a small size, never completely closed. This was visible as a longitudinal slit posterior to the penis-like structure. The urethral opening could be seen just within the vaginal orifice between and at the base of the hypertrophied corpora cavernosa of the clitoris. The corpus cavernosum urethrae was absent so that the organ gave the impression of a hypospadic penis. No change resembling a scrotal sac developed in the skin of the genital zone. The maximum degree of masculinization occurred between 15 and 21 days after the first injection of androgenic substance.

After discontinuing the injections, the genitalia reverted to the normal female type. The initial change in reversal of type was noticeable between 11 and 18 days after the last injection, and the process was completed within 39 to 52 days.

* The testosterone propionate, dissolved in sesame oil and marketed as perandren—Ciba, was supplied by Ciba (China) Ltd., Shanghai.

8 Hu, C. K., and Frazier, C. N., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 116.

The nipples and dewlap were not affected by treatment. In one animal there was a suggestive enlargement of the nipples from which a drop of thin fluid could be squeezed, but the normal female rabbit, especially during pseudopregnancy, which might have been induced in this case, may show the same response. In 2 animals a dewlap was present before the administration of testosterone, but this was not modified along with the masculinization of the genitalia. In the third rabbit, dewlap was not present before treatment and none developed after the injection of testosterone.

Effect on Psycho-sexual Behavior. The sexual behavior of the treated females was remarkably masculine in character. In making this statement we are fully aware of the sexual habits of the rabbit, and of the dual rôle both sexes frequently attempt to play when in the presence of the same sex. With females, the masculinized animals were vigorous and aggressive and made repeated attempts at copulation. One of the treated females was placed successively with 5 normal males, and on each occasion it attempted copulation, playing the part of a male. Only one of the normal males attempted to jump the masculinized female, who sat tight in the cage without moving. The change in sexual behavior was evident 2 weeks after the first dose of testosterone when the clitoris was just beginning to enlarge, and persisted until the external genitalia had returned to the female type. In this connection, Shoemaker[7] observed that the female canary, when treated with testosterone propionate, developed the courtship behavior and singing qualities of the male bird. The masculinizing

Fig. 1.

Ventral view of genitalia showing beginning enlargement of clitoris and swelling of vulva, 2 weeks after the first injection of testosterone propionate.

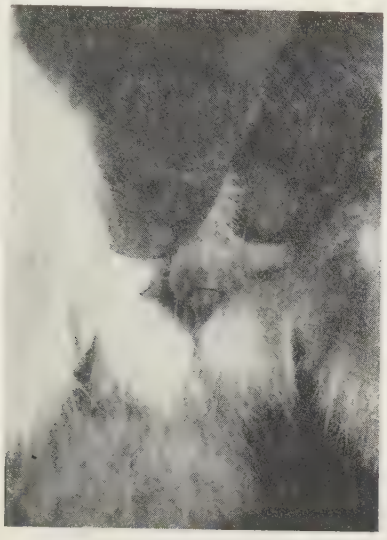

FIG. 2.
Dorsal view of genitalia showing the hypertrophied clitoris and prepuce-like structure, 17 days after the first injection of testosterone propionate.

effect of a testicular graft on the psycho-sexual behavior of the guinea pig was known to Steinach,[9] who reported his observations in 1912.

Effect of Ovariectomy. Ovariectomy made no difference in the response, showing that the effect of testosterone on the lower vaginal zone and clitoris is independent of the ovary. Similar observations were made by Rubinstein and coworkers[10] on the vaginal opening of spayed rats.

The ovariectomized animal, when under the influence of testosterone, showed the same modification in psycho-sexual behavior as had the intact masculinized females.

Summary and Conclusions. Each of 3 adult female rabbits, 8½-15½ months of age, was injected subcutaneously with testosterone propionate, 1 mg per day. Masculinization of the external genitalia followed in about 2 weeks, the clitoris enlarging to form a structure resembling a hypospadic penis, and the labia fusing to partially close the vaginal opening. The anatomical modifications were reversible when treatment was suspended. The psycho-sexual behavior of treated animals became masculine in character. The same response to treatment with respect to somatic sexual characters and psycho-sexual behavior was also induced in a spayed adult female rabbit.

9 Steinach, E., *Pflüger's Arch. f. Physiol.*, 1912, **144**, 71.

10 Rubinstein, H. S., Abarbanel, A. R., and Nader, D. N., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 20.

11063

Immunological Studies on the Cellular Constituents of *C. diphtheriae.*

SAM C. WONG AND T. T'UNG. (Introduced by C. E. Lim.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping.

Since the existence of serological types among *C. diphtheriæ* is well established[1, 2, 3] the isolation of a cellular constituent responsible for type-specificity appears to be possible. Previous reports[4, 5] have shown that the polysaccharides of *C. diphtheriæ* in general are group-specific being shared by members of distinct serological types. From this it may seem obvious that a cellular component other than the polysaccharide is responsible for type-specificity. Because of this a systematic investigation of the various cellular constituents of *C. diphtheriæ* with the object of isolating the type-specific substance was undertaken. On the other hand, it seems also of interest to compare the immunological activities of the various cellular substances.

The organism employed for the present study was the well-known Park 8 strain which belongs to Type D41 of Sia and Huang's serological types.[3] Immune serum was prepared by the intravenous injection of rabbits with well-washed cultures grown on serum-broth, pH 7.6, in accordance with the technic previously used.[4] This will be referred to as the homologous immune serum. Immune sera were also prepared with other serological types of Sia and Huang's such as D25, D30, D40, 6287, 12190, and 2 untyped avirulent strains. This group of immune sera will be designated as heterologous immune sera. Organisms were grown on stomach-digest medium[6] for a week after which they were collected by centrifugation, washed 4 times with distilled water and then with 4 changes of 95% alcohol. The wet bacteria were then suspended in an ether-alcohol mixture (made by mixing equal volumes of each) in the proportion of 20 to 1 respectively contained in a tightly corked flask. The whole mixture was left in the incubator at 37°C for 5 days with frequent agitation

[1] Durand, P., and Guerin, J., *Compt. rend. Soc. de biol.*, 1921, **84**, 980.

[2] Eagleton, A. P., and Baxter, E. M., *J. Hyg.*, 1923, **22**, 107.

[3] Sia, R. H. P., and Huang, C. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 348.

[4] Wong, S. C., and T'ung, T., *Ibid.*, 1939, **41**, 160.

[5] Wong, S. C., and T'ung, T., *Chinese Med. J.*, Suppl. No. 3, 1939, in press.

[6] Young, C. C., *Ibid.*, No. 1, 1936, 143.

during the interim. The supernate was separated from the cells by filtering through ordinary filter paper. The lipoid fraction was obtained by spontaneous evaporation of the solvent and purified by treatment with petroleum ether. The yellow gummy residue obtained was slightly soluble in 95% alcohol but completely in ether and other fat-solvents.

The remaining defatted partially-dried cells were divided into 2 equal portions. One part was extracted with 0.05 N sodium hydroxide while the other was with 1% acetic acid in the proportion of 10 parts of organism to 1 of the extractives. Both extractions were carried out at 37°C with occasional vigorous agitation of the flasks. A few drops of toluol were added as a preservative. The state of cell-autolysis was followed by direct examination of smears made every other day. In the portion treated with alkali, autolysis was found to occur after 3 days of incubation. The fluid, however, was not removed until the 5th or 6th day. The turbid supernate obtained by centrifugation was clarified by filtering through Seitz. The remaining organisms were repeatedly extracted with similar volumes of 0.05 N sodium hydroxide under the above conditions, changes of extractives being made every 5 or 6 days. A total of 3-5 successive changes of extractives sufficed to remove all active substances. To the clear supernate a few crystals of sodium acetate were added as an electrolyte and 1.0 N hydrochloric acid was carefully added until a maximum precipitate occurred. The precipitated protein was collected by centrifugation and washed 3 times with a large volume of sterile distilled water. The precipitate was next suspended in a small volume of distilled water and solution of the precipitate was effected by the slow addition of alkali. Any undissolved material was removed by centrifugation and the solution precipitated once more with acid. The precipitate was again washed thrice with sterile distilled water after which it was suspended in sterile saline. A few drops of alkali were added to dissolve the precipitate and the excess alkali was neutralized with acid. Sterility was obtained by filtering through a sterile Seitz. The total nitrogen was determined by micro-Kjeldahl and the solution was diluted to contain 1% protein. The supernate containing the polysaccharide was precipitated with 3 volumes of 95% alcohol in the presence of sodium acetate. About 5 reprecipitations in 95% alcohol were required to obtain a substance that was easily soluble in 1% solution in saline. The yield for both fractions was comparatively large.

On the other hand, autolysis was found to occur after 7 days in the portion treated with 1% acetic acid but the extractive was not

changed until the 10th day. The supernate was clarified by filtering through a Seitz pad. To the solution was added a few pieces of sodium acetate and one N sodium hydroxide was added slowly until maximum precipitation occurred. The precipitate and the supernate containing the polysaccharide were treated in the same manner as above. The yield for both fractions was comparatively small.

The antigenic activity of the lipoidal, protein, and carbohydrate fractions was determined by the immunization of rabbits, employing 2 animals each. All the animals were first bled and tested for the presence of natural antibodies for the various fractions by the complement-fixation technic. Only those animals whose sera were not anti-complementary and did not contain natural antibodies were used. The immunizing procedure for all the antigens consisted of 3 daily injections followed by 4 days of rest. The process was repeated twice, making a total of 9 injections. Animals were bled 5 days after the last injection. In case of the polysaccharides a total of 9 mg per animal was used while with protein antigens 9 cc of a 1% solution were used. With the lipoid fraction a total of 9 cc of a saturated alcoholic solution diluted 1:4 in saline was employed. The presence of antibodies was determined by agglutination, precipitation, and complement-fixation reactions. In the complement-fixation reaction all immune sera were diluted 1:5 before used. A saturated alcoholic solution of the lipoidal substance was the standard solution upon which various dilutions of the antigen referred in the text were made.

For simplicity of presentation the results are discussed under the following headings:

Lipoid. For the Molisch and protein tests a 1:5 dilution of the lipoid in saline was used. It was found that the lipoid fraction gave a weakly positive Molisch but none of the usual protein reactions. It was non-antigenic and reacted with the homologous immune serum in dilution of 1:160. Approximately similar precipitin-titers were obtained when it was tested with heterologous immune sera. In serological reactions the lipoid fraction is group-specific.

Polysaccharide. It was found that the polysaccharides prepared either by acid or alkali in the absence of high temperature appear to be similar in chemical, serological, and antigenic properties, the total nitrogen for both being 3.1%. Thus the results given for one will be representative of the other. The Molisch reaction was strongly positive when a 1:5000 dilution of the antigen was used and all the usual protein reactions were negative employing a 1% solution. A $1:10^6$ dilution of the polysaccharide reacted with the homologous

serum (ring-test). Approximately the same titer was obtained in similar tests with heterologous immune sera. It was weakly antigenic as shown by the finding that only a complement-fixation titer of 1:50,000 was found when the anti-polysaccharide serum was tested with the polysaccharide. The same immune serum did not give complement-fixation reactions with either the protein or lipoid antigens. No precipitin nor agglutinin could be demonstrated. These results indicate that the polysaccharide is group-specific by serological tests and weakly antigenic in rabbits.

Protein. To the various protein-tests the acid-soluble protein reacted to only the biuret and xanthoproteic although it can be precipitated by ammonium sulfate. Since the fraction is serologically and antigenically inert it need not be discussed further. On the other hand, the alkali-soluble protein reacts with homologous immune serum in dilution of 1:200 (of a 1% solution) but not with heterologous immune sera with a 1% solution. It is weakly antigenic in rabbits giving a complement-fixing titer of 1:500 and agglutinating homologous organism in dilution of 1:10 of the serum. No precipitin could be demonstrated. No cross-reaction was found in complement-fixation reactions when the antiprotein serum (diluted 1:5) was mixed with the polysaccharide or lipoid antigens. This indicates that the antigen is immunologically pure and agrees well with the chemical tests. To show further that the alkaline-soluble protein is type-specific homologous immune serum was absorbed with an organism of the heterologous type. The resulting serum on examination showed that precipitins were completely removed for both polysaccharide and lipoid antigens but not for the alkali-soluble protein.

Since the alkali-soluble protein was found to be type-specific an attempt was made to determine the homogeneity of the protein. Fractionation with various concentrations of ammonium sulfate showed that all the fractions obtained reacted similarly in serological tests, no loss in type-specificity being observed. It is of interest to note that the alkali-soluble protein is heat-labile being destroyed by heating to 56°C for 30 minutes. Protein so treated becomes group-specific, being able to react with homologous as well as heterologous immune sera.

Similar type-specific protein could also be prepared directly from organisms with 0.05 N NaOH at 37°C without previous treatment with ether-alcohol mixture. Such a procedure, however, yielded very little substance even when the time of extraction was extended and the extracting solvent repeatedly changed. Apparently a preliminary

treatment of the organisms with ether-alcohol was necessary in order to render the cells suitable to the extractive action of weak alkali.

Conclusions. A method suitable for the isolation of a type-specific protein in *C. diphtheriæ* is described. Both the lipoid and carbohydrate fractions are group-specific while the alkali-soluble protein is type-specific. The type-specific substance is heat-labile, being converted into a group-specific protein by heating at 56°C for 30 minutes.

11064

Bactericidal Action of X-Rays in the Presence of Dyes.

Chien-liang Hsu and T. Tung. (Introduced by C. E. Lim.)

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Many dyes undergo color-changes when exposed to X-rays or some forms of visible light.[1] This indicates a process of oxidation-reduction. From this and other considerations it is generally assumed that the bactericidal effect of photosensitization is due to the oxidation of microörganisms.[2] It is conceivable that a non-lethal dose of X-rays in the presence of a dye may exhibit a similar function as that shown by visible light. This view is borne out by the following investigation.

Pneumococcus Type I, *Streptococcus hemolyticus, Staphylococcus aureus, B. subtilis, Mycob. phlei* and *Shigella paradysenteriæ* Flexner, *E. typhi, E. coli* and *Pseudomonas aeruginosa* were chosen as the representative gram-positive and -negative microörganisms. Mercurochrome, eosin, methylene blue, crystal violet, and safranin O were employed. The final concentration of mercurochrome and eosin was expressed in term of percent of the dye while that of methylene blue, crystal violet and safranin O, on account of their poor solubility, was expressed in terms of their saturated aqueous solution. The technic previously reported[3] for making the mixture of dye and organisms was followed. In order to expose a number of dye-bacteria mixtures in a limited space accessible to the X-rays, they were put into small glass cups of 1 cc capacity so that 10 of them could be exposed at the same time.

1 Clark, G. L., and Fitch, K. R., *Radiology*, 1931, **17**, 285.

2 Blum, H. F., *Physiol. Rev.*, 1932, **12**, 23.

3 Tung, T., and Zia, S. H., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 326.

The X-rays were produced with a Douglas therapy-tube at 100 KV and 6 MA. The anode of the tube was cooled by an oil-circulating system. With this cooling system the temperature of the irradiated substance never exceeded 38°C. No filter other than the wall of the X-ray tube was used. The distance between the target and the dye-bacteria mixture was 10 cm; 250 r could be delivered to the mixture in one minute. After exposure the exposed and control mixtures were plated and incubated for 48 hours.

X-rays alone in doses of 15,000 r exerted no lethal action on pneumococcus Type I, *Streptococcus hemolyticus, Staphylococcus cureus, B. subtilis, Mycob. phlei, Shigella paradysenteriæ* Flexner, *E. typhi* and *E. coli.* However, when dyes were mixed with the suspensions the same amount of X-rays produced a significant lethal action on some of the microörganisms. This indicates that under suitable experimental conditions X-ray is a powerful germicidal agent. It was found that for gram-positive organisms mercurochrome was the most effective dye in the presence of X-rays. Exposure of pneumococcus Type I and *Streptococcus hemolyticus* to 15,000 r in 60 minutes resulted in complete inactivation of the organisms. Comparative bactericidal tests performed under the same conditions between X-ray-dye-organism and dye-organism system showed that the former was 1000 times more active than the native bactericidal action of the latter alone. The bactericidal action of eosin and methylene blue for these two organisms when exposed to the same amount of X-rays was 100 and 10 times respectively more than their native bactericidal effect. These two dyes, however, produced only slight lethal action on *Staphylococcus aureus* even when the amount of exposure was doubled. Similarly, eosin and crystal violet produced no bactericidal action on *B. subtilis* and *Mycob. phlei* respectively.

Since X-rays in the presence of dyes have exerted a significant bactericidal effect on some of the gram-positive organisms, the question naturally arises as to the possibility on inactivation of gram-negative organisms by the same process. It was found that in using the same dose of X-rays safranin O and eosin exerted a bactericidal effect in 1-100 and 1-10 dilution respectively on *Shigella paradysenteriæ* Flexner, *E. typhi* and *E. coli.* Both safranin O and eosin could kill *Pseudomonas aeruginosa* in 1-10 dilution when 7,500 r were used in half an hour. It may be of interest to mention that doses of X-ray exceeding this value were lethal to this organism in the absence of any dye.

While the photodynamic action on microörganisms could be en-

hanced by the addition of an oxidizing agent[4] the increase of the bactericidal power of X-rays in the presence of dyes could also be demonstrated by a similar treatment. It was found that eosin, after being exposed to 15,000 r in the dilution of 1-100, could not kill *Staphylococcus aureus,* but was able to kill this organism in a dilution of 1-100,000 when 1% hydrogen peroxide was introduced into the dye-bacteria mixture. Control experiments showed that eosin together with hydrogen peroxide in the absence of X-rays or hydrogen peroxide in the presence of X-rays did not produce any bactericidal effect.

In comparing the results of the present study with those of the previous investigation[3] on the photodynamic action of various dyes on microörganisms, certain similarities and differences can be observed. In the first place, pneumococcus Type I is the most susceptible organism to both photoinactivation and the combined action of X-rays and dyes while *Staphylococcus aureus,* spore-bearing and acid-fast organisms were the most resistant. In the second place, the bactericidal power of eosin in conjunction with either visible light or X-rays can be greatly increased by the addition of an oxidizing agent. On the other hand, eosin was found to possess little or no photodynamic activity on gram-negative organisms and yet in the presence of X-rays it exerted distinct bactericidal effect on organisms of the dysentery-typhoid-colon group. Similarly, eosin and safranin O which were proved to be potent photodynamical substances on gram-positive and -negative organisms respectively when exposed to visible light, were found to exert a weaker bactericidal action in the presence of X-rays.

The fact that a non-lethal dose of X-rays exerted bactericidal action in the presence of dyes suggests that the process involved is analogous to that of photodynamic action. It is to be noted, however, that, with the present amount of X-rays, the germicidal effect of the former is inferior to that of the latter.

Summary. Mercurochrome, eosin, and methylene blue exerted a bactericidal action on gram-positive organisms concurrently exposed to X-rays. While eosin is not a photodynamically active substance on gram-negative bacteria it produced a distinct bactericidal action on them in the presence of X-rays. The facts that a non-lethal dose of X-rays produced germicidal action in the presence of dyes and that such an action could be enhanced by introducing an oxidizing agent suggests that the fundamental mechanism of photosensitization and the combined action of X-rays and dyes may fall into the same category.

[4] Tung, T., Proc. Soc. Exp. Biol. and Med., 1938, **39**, 415.

11065

Effect of Frozen-Dried Plasma and Frozen-Dried Embryo Juice on Tissue Cultures.

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In view of the remarkable results obtained in keeping bacterial antigens, sera and other products without alteration for long periods of time, following freezing and drying *in vacuo*, it was undertaken to determine whether plasma and embryo juice for tissue culture could also be treated similarly. The following report describes briefly the procedure and the results obtained.

Young chickens were deprived of food for 24 hours and then bled aseptically by cardiac puncture. Not more than 20 cc of blood was removed at a time. It was prevented from clotting by being drawn into syringes moistened with a solution of heparin. The plasma was collected after centrifuging the blood on ice, and was pooled before distribution in one cc quantities in small glass ampoules prior to freezing and drying with a Lyophile* apparatus. The ampoules sealed *in vacuo* were labeled and stored in an icebox.

Twenty percent embryo juice was prepared in the usual manner,† using Ringer-Tyrode solution (glucose 0.2%) and chick embryos of 11 days' incubation. This material in one cc quantities was likewise frozen, dried, sealed *in vacuo* and stored. To determine whether these 2 products would serve as adequate media for tissue cultures, the following experiment was performed.

The ampoules of dried plasma and embryo juice which had been kept in storage for 3 months were filed, sterilized in alcohol, and wrapped in sterile gauze before breaking the necks to open them. The dried powders were dissolved and diluted to original volume with sterile, triply distilled water. The plasma always dissolved without residue but a few flecks of insoluble material remained in the embryo

* The apparatus and method of freezing and drying the plasma and embryo juice were those described by Flossdorf, Earl W., and Stuart Mudd, *J. Immunol.*, 1935, **29**, 389.

† Twenty per cent embryo juice was made by weighing out 10-day embryos in a sterile dish and adding for every gram, wet weight, 4 cc of Ringer-Tyrode solution. The embryos were minced with sharp scissors and the mixture centrifuged at high speed. The supernatant fluid constituted the juice which was then frozen and dried *in vacuo*.

TABLE I.

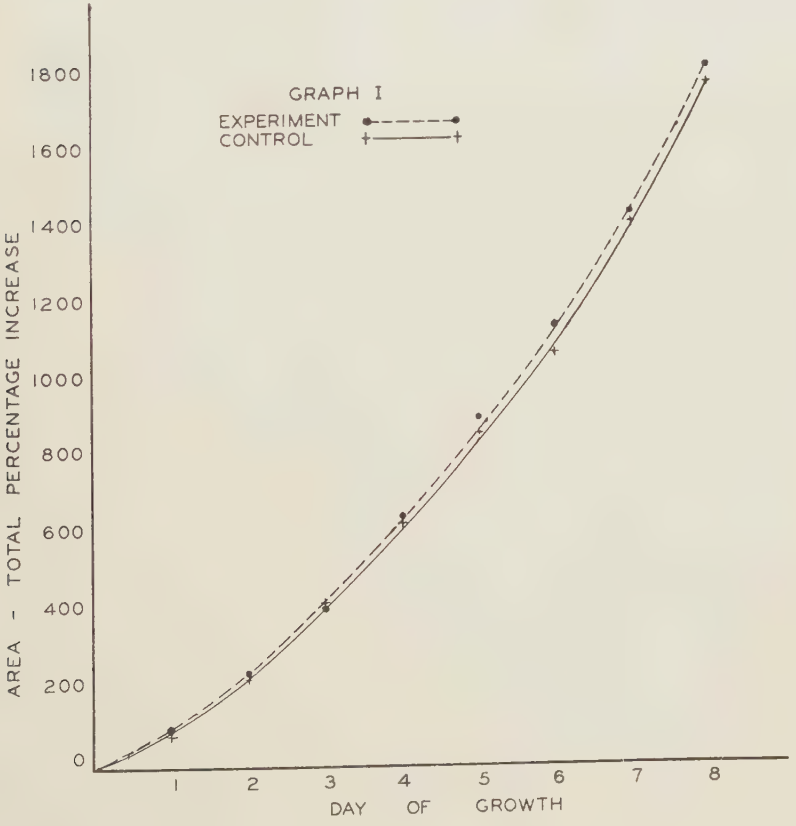
Days of growth	Control Series: Mean total area, mm²	Probable error of mean	Standard deviation	Total growth, %	D_M*	σ_D	$3\sigma_D$	Significant difference	Experimental Series: Mean total area, mm²	Probable error of mean	Standard deviation	Total growth, %
0	0.63	±.01	0.20	0	0.12	0.03	0.09	+	0.75	±.02	0.24	0
1	1.19	±.04	0.48	84	0.32	0.09	0.27	+	1.51	±.05	0.66	101
2	2.12	±.06	0.83	236	0.48	0.15	0.45	+	2.60	±.08	1.10	246
3	3.32	±.10	1.39	427	0.52	0.26	0.78	0	3.84	±.14	1.99	412
4	4.63	±.14	1.97	634	1.01	0.35	1.05	0	5.64	±.19	2.63	652
5	6.11	±.18	2.55	869	1.51	0.46	1.38	+	7.62	±.25	3.48	916
6	7.44	±.24	3.30	1080	1.90	0.58	1.74	+	9.34	±.31	4.28	1145
7	9.60	±.32	4.36	1423	2.01	0.73	2.19	0	11.61	±.38	5.16	1448
8	11.88	±.38	5.21	1785	2.60	0.87	2.61	0	14.48	±.45	6.12	1830

*D_M, difference of the means; σ_D, standard error of the difference of the means; significant difference = $D_M > 3\sigma_D$.

juice. Cultures of 11-day chick embryo hearts were planted in equal parts of the experimental plasma and embryo juice. From portions of the same hearts, control cultures in equal numbers were planted in freshly-drawn plasma and freshly prepared 20% embryo juice. The hanging-drop, cover-slip method was used and all cultures were incubated at 37.5°C.

Delineascope and planimeter were used to measure the areas of the original explants. During the succeeding 8 days the total area of each culture was measured at 24-hour intervals. The results were treated statistically and appear in Table I. In each series there were about 110 cultures, and from each of these groups the records of the first 85 cultures in full growth at the end of 8 days were tabulated.

It is apparent from Table I and Graph 1 that the growth of the experimental cultures and of the controls was almost identical. Furthermore, careful microscopical study of the living cells revealed no discernible difference between those growing in the new media as compared with those in the standard media.



The simplicity of planting cultures using frozen-dried plasma and embryo-juice is particularly attractive when technical help is limited, since much routine of preparation can be eliminated. The only disadvantage so far experienced with the plasma is that clotting time is slightly delayed.

11066 P

Mechanism of the Therapeutic Effect of Metrazol and Insulin Convulsions.*

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Anoxemia has been regarded as a common factor in the effects of insulin and metrazol convulsions, in that hypoglycemia diminishes the oxygen utilization of brain tissue (Holmes,[1] Wortis[2]), while the metrazol convulsions interfere with the respiratory movements (Himwich[3] and coworkers). Anoxemia may act by stimulating the sympathetic system (Gellhorn[4]), it may, however, affect the brain cells directly by increasing the permeability of the cellular surface films (Spiegel and Spiegel-Adolf[5]). The question may, therefore, be raised whether insulin and metrazol convulsions change the permeability of the cells of the central nervous system.

In 10 guinea pigs metrazol convulsions were produced (2 cc metrazol† intraperitoneally), in 10 others insulin convulsions (20-40 units of insulin). The brains of 3 guinea pigs were studied after the animals had received insulin, but before the onset of convulsions, while the measurements on 10 further animals served as normal controls. Part of the animals were killed by decapitation during the

* Aided by a grant from the American Medical Association, Committee on Scientific Research.

1 Holmes, E. G., *Biochem. J.*, 1930, **24**, 914; 1932, **26**, 2010.

2 Wortis, S. B., *New York State J. of Med.*, 1938, **38**, 1015.

3 Himwich, H. E., Bowman, K. M., Wortis, J., and Fazekas, J. F., *J. Am. Med. Assn.*, 1939, **112**, 1572.

4 Gellhorn, E., *Arch. Neur. and Psych.*, 1938, **40**, 125.

5 Spiegel, E., and Spiegel-Adolf, M., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 799; *J. Nerv. Ment. Dis.*, 1939, **90**, 188.

† The metrazol was kindly supplied by the Bilhuber-Knoll Corp.

convulsions (minimal duration of the convulsions 5 minutes), in the remainder the convulsions or the insulin "shock" were allowed to continue until the spontaneous death of the animal. The polarizability and thus indirectly the permeability of the cell surfaces was determined 5-10 minutes postmortem by measuring the conductivity at high (C_h) and at low frequencies (C_l) on the exposed cerebral hemispheres at 37°C temperature and calculating the polarization index $\Delta = 100(C_h - C_2)/C_2$ (Spiegel and Spiegel-Adolf[5]). The frequencies used in this study were 5120 and 547 respectively. In some animals the brain was used for histological studies after the measurements; besides, the brains of animals subjected to metrazol or insulin convulsions as described above, were histologically studied without being altered by the electrical measurement. The brains were fixed in alcohol and stained with toluidine blue and hematoxylin respectively.

The normal values of the polarization index of the cerebral hemispheres varied between 11.9% and 17.8% (mean 13.75 ± 0.37). The brains of animals subjected to metrazol convulsions showed values ranging between 9.4% and 12.9% (mean 11.44 ± 0.22). The influence of insulin convulsions is still more marked, the Δ values after these convulsions ranging between 8.4% and 12.3% and giving a mean of $10.84\% \pm 0.24$. The difference between the normal and the metrazol series is 2.31 with a probable error of ± 0.43, that between the normal and the insulin series 2.91 with a probable error of ± 0.44.‡ That these differences are significant could also be shown by applying the t-test (see Fisher[6]). A comparison of the means of the normal and of the metrazol series yields $t = 3.42$, comparison of the normal and insulin series $t = 4.18$. The corresponding values of P (probability of falling outside the range $\pm t$) are below 0.01, indicating that the differences may be considered as highly significant from a statistical point of view. Control experiments in which animals received the same amount of insulin, but were killed before the onset of the convulsions, showed that this depression of the Δ did not become manifest before the convulsions developed.

Thus metrazol and insulin convulsions, the latter somewhat more than the former, distinctly lower the polarizability of the cerebral hemispheres; this finding indicates that these convulsions increase the

‡ We are indebted to Dr. S. Peller (Department of Biology, Johns Hopkins University School of Hygiene) for his help in the statistical analysis of our data.

6 Fisher, R. A., *Statistical Methods for Research Workers*, 7th edition, Oliver & Boyd, London, 1938.

permeability of the cellular surface films. In general, the changes of Δ are the least pronounced after convulsions of short duration and the most marked after long lasting convulsions, as particularly shown by the insulin animals. It seems, however, that the duration is not the only factor determining the degree of permeability change, since some cases do not follow this rule. The histological controls may only be briefly mentioned since they chiefly confirm findings reported in the literature.[7] In agreement with the electrical measurements they showed that the nerve cells of the cerebral cortex were more affected by the insulin than by the metrazol convulsions. Particularly in the deep cortical layers cells were found with pale staining, partial (perinuclear) or total tigrolysis, occasionally cell shadows, or vacuolation of the protoplasm. Rarely some cells were more darkly stained than normally. The nuclei were usually well preserved, only occasionally homogeneous shrinking or peripheral position of the nucleus were observed. All these changes were, as mentioned, more marked in the insulin animals than in the metrazol animals. The architecture of the cortex was preserved in all animals. Hemorrhage could not be found.

The increase of the cellular permeability produced by insulin and metrazol convulsions may perhaps contribute to an understanding of the therapeutic effects of such convulsions, in that the decrease of the density of the cellular surface films facilitates the exchange of ions between the cytoplasm and its environment and the removal of products of its metabolism. It is also conceivable that repeated injuries of the cell membrane may eventually result in more severe lesions of the cells as shown by the histologic examination after prolonged convulsion treatment.

[7] Weil, A., Liebert, E., and Heilbrum, G., *Arch. Neurol. and Psychiat.*, 1938, **39**, 467; Strecker, E. A., Alpers, B. J., Flaherty, J. A., and Hughes, J., *Arch. Neurol. and Psychiat.*, 1939, **41**, 996.

11067 P

Hypoglycemia and Increased Insulin Sensitivity Following Hypothalamic Lesions.*

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An occasional demonstrable fall in the blood sugar level, in the dog, following traumatic transverse lesions in the hypothalamus at the level of the optic chiasm was observed by us[1] (see 1, Fig. 1a). Later profound hypoglycemic crises were encountered, also occasionally, when the ventral third or half of the hypothalamus was severed free by a sweeping semicircular cut made with a small blunt instrument[2] (see 2, Fig. 1a). Subsequently Ingram and Barris described persistent mild hypoglycemia and increased insulin sensitivity following bilateral lesions placed in the region of the paraventricular nuclei in the cat.[3]

The question as to the mechanism involved in the precipitation of hypoglycemia, particularly the crises, has attracted our active attention. Below are interpretations drawn from the results obtained thus far in the investigation. The results pertain to experiments on a large series of dogs.

The possibility of the hypothalamic lesions eliminating a normally tonic descending innervation (brainstem) is ruled out on the basis of the following observations: (1) hypoglycemia was precipitated only occasionally by what appeared to be identical lesions; (2) in no instance was hypoglycemia precipitated by lesions that severed the dorso-caudal hypothalamic connections (see 3, Fig. 1b); and (3) crises occurred characteristically in the presence of bilateral vagotomy as well as bilateral abdominal sympathectomy.

The possibility of a descending (brainstem) innervation being activated, either by release or by irritative effects of the lesions, is tentatively ruled out by the failure of vagotomy or sympathectomy to protect against the crises. However, the crucial experiment in this respect has not been carried out, namely, experiments involving both vagotomy and sympathectomy.

* Aided by a grant from Rockefeller Foundation.

1 D'Amour, M. C., and Keller, A. D., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 772.

2 Keller, A. D., Noble, W., and Keller, P. D., *Am. J. Physiol.*, 1935, **113**, 79.

3 Ingram, W. R., and Barris, R. W., *Am. J. Physiol.*, 1936, **114**, 562.

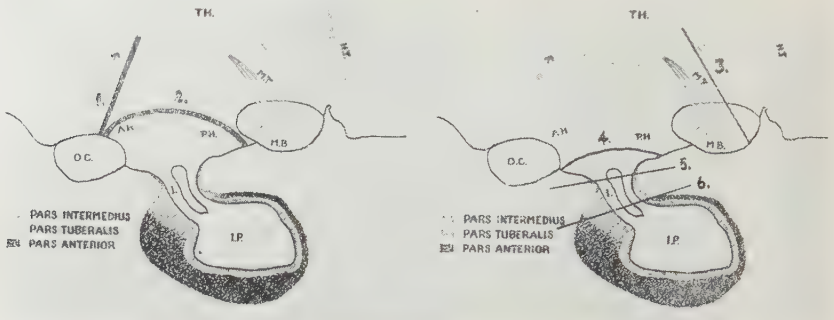

FIG. 1.

Schemata of the hypothalamus and hypophysis of the dog. For explanation of procedures 1 to 6 see text. o.c., optic chiasm; f., fornix; a.h., anterior hypothalamus; p.h., posterior hypothalamus; th., thalamus; m.t., mammillothalamic tract; m.b., mammillary body; h.t., habenular tract; i., infundibulum; and i.p., infundibular progress.

The possibility of the hypothalamic lesions eliminating a normally tonic hypothalamico-hypophysial innervation is ruled out on the basis of the failure of hypoglycemia to follow the separation of hypophysis from the hypothalamus by the following procedures, (1) section of the hypophysial stalk at any level,[4] (see 5 and 6, Fig. 1b), and (2) severing all hypothalamico-hypophysial nerve fibers as they pass through the extreme ventral portion of the hypothalamus, *i. e.*, before their entrance into the infundibulum, (see 4, Fig. 1b). In instances these procedures have been accomplished *with no change in insulin sensitivity,* however, a persistent milk increase (from 2 to 4 times) is exceedingly prone to result.†

Assuming that these experiments were not complicated by as yet unrecognized factors, there remains the possibility that hypoglycemia and increased insulin sensitivity following hypothalamic lesions is precipitated either by the activation of a normally atonic hypothalamico-hypophysial innervation, or by the *derangement of a purely hypophysial mechanism,* induced by immediate neighboring traumatic hypothalamic procedure. The possibility of an endocrine factor common to both the hypophysis and hypothalamus should also be borne in mind.

[4] Keller, A. D., Noble, W., and Hamilton, J. W., *Am. J. Physiol.*, 1936, **117**, 467.

† In the cat increase in insulin sensitivity following procedures 4 and 5 has ranged from 2 to 20 times. Typical marked obesity with no change in insulin sensitivity has been encountered in several instances in the dog following procedure 4.

11068

Correlations between Epidermal Impedance and the Clinical Course in Certain Psychoses.

A. Barnett. (Introduced by C. Landis.)

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No objective, experimental method has, as yet, been described for accurately estimating the clinical level in mental disease. Although the outward manifestations of a swing from catatonic stupor to catatonic excitement in a schizophrenic or from a manic to a depressed state in a cyclothemic may be very striking from the standpoint of an observer, we remain in comparative ignorance of the basic processes producing these changes. The purpose of the present paper is to describe a simple, objective, quantitative method which appears to follow variations in the clinical state in certain types of mental disease with considerable fidelity and which, at the same time, shows promise of throwing light on the underlying processes responsible for the changes observed.

In the course of impedance measurements carried out on all of the adult mental patients (about 125) hospitalized at the N. Y. State Psychiatric Institute and Hospital, it was observed that when certain patients became violent, markedly disturbed or confused, necessitating transfer to the agitated wards, the skin impedance values showed a decrease which in many cases was very marked and that, contrariwise, when the same patients calmed down or improved sufficiently to permit transfer back to a non-agitated ward, the measured impedances showed a counter-swing towards and even above their original levels. It was observed, moreover, that the patients exhibiting this phenomenon fell into no specific diagnostic group, catatonics, cyclothemics, involutionals and even organic psychoses each contributing their quota.

The evolution of the impedance values in 7 of these cases is shown in the accompanying figure. Measurements of the epidermal impedance were made on the posterior and anterior surfaces of the right upper arm by the 3-electrode method at 11,160 cps, as previously described,[1, 2] using an annulus having having a surface area of 6 cm^2. The means of the posterior and anterior values are plotted in the various curves.

1 Barnett, A., *J. Physiol.*, 1938, **93**, 349.

2 Barnett, A., *West. J. Surg.*, 1937, **45**, 540.

All of the cases shown, although of widely varying clinical character, present essentially the same features in the evolution of the impedance curves; (1) during periods of increasing excitement, agitation or resistiveness, the curves descend; (2) during periods of depression, quiet or recovery, the curves ascend.

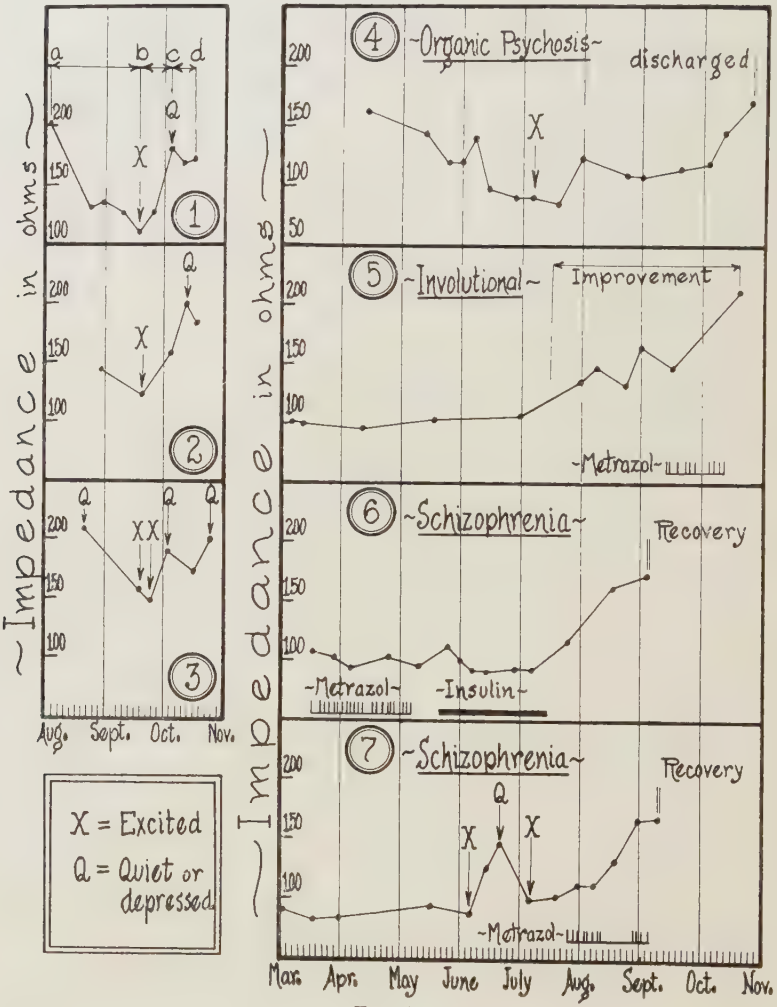

FIG. 1.

Correlations between epidermal impedance and the clinical course in mental disease.

Ordinates, impedance in ohms. Abscissæ, time in days, each subdivision represents a 3-day period. The electrode area is 6 cm².

The numbers in each rectangle correspond to the case numbers given in the text. In cases Nos. 5, 6 and 7, the number and spacing of the metrazol treatments is indicated by a short vertical line on each day of treatment.

An analysis of case No. 1 will serve to illustrate these changes. This patient, presenting a pure manic-depressive syndrome accompanying the menopause was admitted in a relatively quiescent state. During the 3 weeks following, she became more and more excited, talking and shouting constantly. These changes were reflected in the impedance curve by descending branch *a-b*. Very suddenly and at the height of the period of excitement, the patient entered a quiet phase, *b-c*, in which she spoke in reserved tones and conducted herself in a perfectly normal manner. The impedance curve moved upward during this period to plateau level *c-d*, where it remained until her discharge.

Cases No. 2 and No. 3 present a similar parallelism between the course of the impedance curves and the clinical state. The diagnosis in case No. 2 (male, age 16) was hesitant as between an early schizophrenia (catatonic) and cyclothemia. In case No. 3 (male, age 26), a history of chronic purulent otitis and findings of marked variability in the pressure and the protein content of the spinal fluid associated with suggestive neurological signs made the diagnosis undecided as between an organic psychosis (possible brain abscess) and schizophrenia (catatonic). Both of these cases were in full clinical evolution at the time of the present writing.

Cases No. 4 to No. 7, inclusive, are given to show clinical-impedance correlations where changes in the clinical course take place over a protracted period.

Case No. 4 (male, age 33) is that of a Parkinsonian with a psychosis having the characteristics of a catatonic schizophrenia. It may be regarded as organic. This patient presented a gross tremor of the right hand and arm exaggerated by intentional movements and a marked hypertonus of the muscles of the leg on the same side (cog-wheel rigidity). The psychosis included hallucinatory, confusional, negativistic and excitatory symptoms which, beginning in mid-April, rose to a maximum early in July, at which time the patient had to be tube fed and would remain standing in one spot for hours in a position of extreme opisthotonus. Improvement took place along the ascending branch of the impedance curve until the time of his discharge, incompletely recovered, in late October. The impedance curve, here, is an excellent representation of the clinical course.

Case No. 5 (female, age 53) is an involutional melancholia in which symptoms of agitation predominated. The clinical state as well as the impedance curve remained at a substantially stationary level from March to July. This was followed by a moderate spon-

taneous improvement over the period of the next 2 months which permitted transfer of the patient from an agitated to a non-agitated ward. A series of metrazol treatments were then given which resulted in further and more rapid improvement. The impedance curve, here again, reflects very well the clinical course.

Case No. 6 (female, age 39) is a schizophrenia (catatonic) in which the clinical state as well as the impedance remained at a stationary level over a period of about 4 months, during which time there were frequent outbreaks of violence. A course of metrazol treatments given during this period had little effect and was followed by insulin shock treatment. Some improvement became apparent near the end of the insulin series and accentuated itself during the month following. The upward course of the impedance curve follows the clinical improvement with considerable fidelity.

Case No. 7 (female, age 33), a schizophrenia (catatonic-paranoid), shows an impedance curve with a peaked portion occurring in the month of June. A marked improvement was observed at the maximum of this peak, but shortly thereafter, the patient's condition reverted to its former state (downward portion of curve) with signs of deterioration (playing with feces, smearing of food on arms and face). A course of metrazol was then given with progressive improvement and recovery as shown by the upward course of the impedance curve during August and early September.

Because of the fact that the total impedance change may cover a range of as much as 100% (case No. 4), measurements of the phase angle of the skin were also made[1] in each case to determine whether any marked variation in the ratio between the reactive and resistive components accompanied the change in impedance. All of the phase angle values fell between 69° and 75° and the maximum change in any case was 4°, corresponding to a variation in the ratio between the reaction and resistive components of only 2-3%.

Finally, as a cross-check on the epidermal impedance measurements, Q-factor readings were taken on the arm-to-arm segment of each patient both by the immersion[3] and 4-electrode methods.[4, 5] Q-factor determinations by the immersion method include a skin component contributing about 60% of the total reactance[3, 5] and changes in skin impedance would show up in the total Q-factor measurement, provided the variations in the Q of the deep tissues remained small.[4] Whenever this was found to be the case, for each change of

[3] Barnett, A., *West. J. Surg.*, 1937, **45**, 380.

[4] Barnett, A., *West. J. Surg.*, 1937, **45**, 612.

[5] Horton, J. W., and Van Ravenswaay, A. C., *J. Frankl. Inst.*, 1935, **220**, 557.

epidermal impedance, a corresponding shift in the Q-factor (immersion) was found to take place.

Discussion. The findings here reported are of a preliminary nature and do not permit a judgment, as yet, as to how generally impedance readings of this type reflect changes in the clinical state. The cases selected for presentation were chosen, largely, because they included an agitated phase and the clinical changes were so marked in character as to admit of no doubt in the minds of a number of observing clinicians. Whether or not, as a result of further studies, impedance readings be found to follow changes in the clinical state as a general rule, the group of cases here presented may be considered to constitute evidence for the existence, in certain mental patients, of an impedance syndrome characterized by a parallel evolution of the epidermal impedance values and the clinical course. This syndrome may be separated from the general body of the psychoses for special study and may throw considerable light on the mechanisms responsible for this particular clinical entity.

The relative constancy of the phase angle of the skin during an impedance variation of as much as 100% rules out any possibility of the direct current resistance of the skin being responsible for the impedance change. Impedance changes are best explained as representing corresponding variations in thickness of the epidermis.

Summary. In certain forms of mental disease, the epidermal impedance is found to reflect variations in the clinical course, the values decreasing during phases of agitation and increasing during periods of quiet, depression or recovery. The phenomenon is not limited to any diagnostic group and may be found among catatonics, cyclothemics, involutionals and even in the organic psychoses.

11069

Acute Toxicity of Mononitrobenzene in Mice.

MICHAEL B. SHIMKIN. (Introduced by H. L. Stewart.)

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In a comprehensive review of nitrobenzene intoxication, covering 265 papers, Schneider[1] states that "the minimal toxic dose in animal experiments is nowhere noted." The investigations on dogs, cats, rabbits, guinea pigs, rats[1] and frogs[2] are concerned primarily with the manifestations and effects of mononitrobenzene poisoning, and with the study of methemoglobinemia or, as in guinea pigs[3] and rabbits[4] some other non-oxygen-carrying derivative of hemoglobin formed with the agent.

The minimal lethal dose of mononitrobenzene (oil of mirbane) was determined in mice in the course of experiments on its effect upon tumor growth. The report was stimulated because mice have not been generally used in the studies of this important industrial poison, and because the toxic manifestations in mice were practically identical with those described in man.[1, 2, 5]

Experimental: Eighteen female mice of the C_3H strain, weighing 35-40 g, were shaved over the abdomen and were painted lightly with a brush soaked in mononitrobenzene over an area less than one-tenth of the body surface. In one hour, 15 mice were in partial collapse, but all recovered within 24 hours. The animals were painted again; next day, 3 were dead. The rest were painted a third time; within 48 hours, 9 died. Thus, after 3 applications, 24 hours apart, the mortality was 12 out of 18 animals.

Ten male mice of strain A, about 30 g in weight, were painted vigorously over the unshaved abdomen for about 20 seconds. In 30 minutes, all were in partial collapse, and in one hour, 6 were motionless, cold and breathing forcibly; 2 were dead within 3 hours, and 4 within 20 hours. Two more mice, which apparently had recovered

1 Schneider, W., *Veröffentlich. a. d. Gebiete. Medizinalverwaltung,* 1933, **39**, 585.

2 Mancini, M. A., and Guidi, G., *Arch. Internat. Pharmacodynamie et Therapie,* 1922, **26**, 247.

3 Levin, S. J., *J. Am. Med. Assn.,* 1927, **89**, 2178.

4 Stimson, B. B., *J. Biol. Chem.,* 1927, **75**, 741.

5 (a) Adams, S. S., *Tr. Assn. Am. Physicians,* 1912, **27**, 503; (b) Stifel, R. E., *J. Am. Med. Assn.,* 1919, **72**, 395; (c) Hamilton, A., *Industrial Poisons in the United States,* Macmillan, N. Y., 1925, p. 497.

TABLE I.
Toxicity of Mononitrobenzene Subcutaneously.
(Sesame oil dilutions 1:5 and 1:10.)

Mononitrobenzene in sesame oil cc	No. mice used	Died	
		No.	Time in hr
.1	3	3	3-12
.05	10	10	6-18
.025	10	10	24-48
.010	15	2	96-120
.005	10	0	—
.001	9	0	—

from collapse, died within 3 days; the total mortality, therefore, was 8 out of 10 animals.

To determine the amount of mononitrobenzene absorbed, graded doses were dissolved in sesame oil in 1:5 and 1:10 dilutions, and were injected subcutaneously into mice. As summarized in Table I, the minimal lethal dose was about 0.01 cc, or 0.0004 cc per gram of body weight.

Symptoms and Signs: About 30 minutes after receiving the compound percutaneously or subcutaneously, the mice were prostrated and lay motionless or with occasional twitching movements. They were cold and the respirations were slow and labored. Some of the animals recovered from this collapse in one to 3 hours and remained well; others remained completely motionless and cold, with extremely slow respiration, for over 24 hours before they died. Death was due to respiratory failure; the heart continued to beat many minutes after the cessation of breathing.

One to 3 hours after exposure to mononitrobenzene the skin developed a dark grey-blue hue, and the blood became chocolate-colored and more viscous than normally. Spectroanalysis of the blood revealed a strong absorption band at 6350 Å (methemoglobin).[6] The urine was orange in color and had a strong odor of mononitrobenzene.

Three hours after the application of the compound the white blood cell count dropped from the normal 11,000 to 14,000 per cmm to 5,000 per cmm, with a normal differential count. The red blood cell count was not affected, remaining over 9 million per cmm. Twenty-one hours later, when the animals were moribund, the white blood cell count was 1000 to 1500 per cmm or lower, with a normal differential count (PMN, 30%, with 6% nonfilaments and 3% metamyelocytes;

[6] (a) Heubner, W., *Zentralbl. f. Gewerbehygiene*, 1914, **2**, 409; (b) Hamilton, A., *Industrial Poisons in the United States*, p. 493.

lymphocytes, 60%; monocytes, 4%).[7] The red blood cell count was 7 to 8 million, and the smears showed hypochromia and hemolysis. The methemoglobinemia precluded routine hemoglobin determinations.

Necropsy Findings: All animals that died or were killed when moribund had the same pathological findings: dark grey-blue skin, dark chocolate colored blood, orange urine with a strong mononitrobenzene odor; the livers were white and soft, some having only small areas of normal red tissue, and others having latticed white areas throughout the organ. Other organs were grossly normal.

Histologically, the most marked lesion was in the liver.[8] There was diffused necrosis of the outer portions of the liver lobules, sometimes involving two-thirds of the lobules. The cell outlines were still visible but the cytoplasm was pale and granular, and many of the nuclei had completely disappeared. There was a large amount of dark, brownish pigment in the Kupffer cells; the pigment was more prominent in the necrotic portions of the lobules. No notable changes were seen in the bile ducts. The vessels and the sinusoids contained numbers of red blood cells which were pale, and very few white blood cells. The kidneys showed slight swelling of the glomeruli and of the tubular epithelium. No morphologic changes were observed in the spleen, lungs, or testes.[9]

Effect on Tumors: Sublethal doses of mononitrobenzene, administered every other day either by painting or by subcutaneous injections diluted in sesame oil, failed to influence the growth of cutaneously transplanted sarcoma 37 in 15 strain A back-cross mice, or in 8 strain C_3H females bearing spontaneous mammary carcinomas. The absorption of mononitrobenzene is apparently more effective over highly vascularized areas. Ten C_3H females with spontaneous breast tumors were divided into 2 groups: in 5, the compound was painted over the shaved abdomen and in 5 over an approximately equal area of the skin covering the tumor. In 24 hours, all of the former had recovered, while all of the latter group were moribund.

Acknowledgment is due to Dr. F. T. Hunter for the spectroanalysis of the blood, and to Dr. H. G. Grady for review of the histologic material.

7 Carter, F. W., *Med. J. Australia*, 1936, **2**, 558.

8 Leader, S. D., *Arch. Pediat.*, 1932, **49**, 245.

9 Dresbach, M., and Chandler, W. L., PROC. SOC. EXP. BIOL. AND MED., 1918, **15**, 136.

11070

Comparison of Certain Pharmacological and Antibacterial Properties of p-Hydroxaminobenzenesulfonamide and Sulfanilamide.*

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Mayer[1, 2, 3] advanced the hypothesis that p-hydroxaminobenzenesulfonamide was responsible for the antibacterial action of sulfanilamide. In support of this theory, Mayer found (1) that the hydroxylamine‡ *in vitro* was 100 times more bactericidal than sulfanilamide and acted without a latent period; (2) that it converted hemoglobin to methemoglobin, thus explaining the frequent appearance of this pigment in the blood of patients treated with sulfanilamide, and (3) that para-nitrobenzene sulfonamide was inactive *in vitro* but 5-6 times more active than sulfanilamide *in vivo* (the nitro-compound being presumed to yield hydroxylamine more readily than the amine). The hydroxylamine was found to be no more active than sulfanilamide in the mouse infected with streptococci, but Mayer considered that this was due to its rapid decomposition by the blood pigment and that conditions were quite different when hydroxylamine was formed from the amino- or nitro-compound in the immediate vicinity of the bacteria.

Recently, considerable interest has been manifested in the hydroxylamine theory of sulfanilamide action.[4, 5, 6] Methods of preparation and properties of the hydroxylamine and certain of its derivatives, a method for its analysis, the stability of its aqueous solutions, its activity *in vitro*, and its behavior in animals are presented in this communication.

Preparation and Properties. Since the method of preparation and

* This investigation has been aided by a grant from the John and Mary R. Markle Foundation.

† Lalor Foundation Fellow.

1 Mayer and Oechslin, *Compt. rend. Acad. d. sc.*, 1937, **205**, 181.

2 Mayer, *Bull. Acad. de méd.*, Paris, 1937, **117**, 727.

3 Mayer, *Biol. Med.* (supplement), 1937, **27**, 74.

‡ For the sake of brevity, p-hydroxaminobenzenesulfonamide will be referred to as the hydroxylamine.

4 Locke, Main and Mellon, *Science*, 1938, **88**, 620.

5 Shaffer, *Science*, 1939, **89**, 547.

6 Rosenthal and Bauer, *Public Health Reports*, 1939, **54**, 1880.

properties of p-hydroxaminobenzenesulfonamide have not been described, they are included here. Starting with p-nitrochlorobenzene, p,p′-dinitro-diphenyldisulfide was prepared,[7] using 2 moles of sodium disulfide. This disulfide may be converted to p-nitrobenzenesulfonamide by either of 2 methods: (a) oxidation to ammonium p-nitrobenzenesulfonate[8] with subsequent formation of the acid chloride, then the amide by the usual procedure;[9] (b) direct conversion of the disulfide to the acid chloride by chlorine in the presence of nitric acid,[10, 11, 12] followed by conversion to the amide. The yield of amide by method (a), based on p-nitrochlorobenzene, was 47%; by method (b), 35%.

Ten grams of p-nitrobenzenesulfonamide was ground intimately with 7.2 g of zinc dust (90%). This was added during 10 minutes with moderate stirring and appropriate cooling to 100 cc of water maintained at 65°C, containing 2.7 g of ammonium chloride. Stirring was continued 10 minutes longer. The mixture was quickly filtered by suction, and the zinc oxide cake washed with two 15 cc portions of hot water, the washings being received in the filtrate. Thirty grams of C.P. sodium chloride was added to each 100 cc of filtrate, the flask evacuated, and rotated in a tilted position in an ice bath for 45 minutes. The crude hydroxylamine was filtered by suction onto a 12 cm Büchner funnel and sucked as dry as possible, using rubber dam on the funnel to minimize oxidation. The precipitate was dried overnight over calcium chloride in a well-evacuated desiccator in the ice chest. The crude hydroxylamine was boiled with 3 successive 400 cc portions of anhydrous C.P. ether, filtering each extract into an equal volume of petroleum ether. After chilling, the precipitates were collected separately and dried *in vacuo*. Yield, 1.0-1.5 g (10-16%).

The hydroxylamine is a white powder, which under the microscope appears as colorless irregular prisms. It melts at 139.5-140.5°C with slight decomposition.§ Its solubility at 25° in various solvents is: water, about 2%; absolute alcohol, about 5%; absolute ether, about 0.2%. It is soluble in alkali and dioxane, moderately soluble in acetone, slightly soluble in ethyl acetate, very slightly solu-

7 *Organic Syntheses*, 1928, **8**, 64, John Wiley & Sons, New York.

8 Wohlfahrt, *J. prakt. Chem.*, 1902, (2) **66**, 553.

9 Obermiller, *J. prakt. Chem.*, 1914, (2) **89**, 85.

10 Fierz, Schlittler, and Waldmann, *Helv. chim. Acta*, 1929, **12**, 667.

11 *Organic Syntheses*, 1935, **15**, 55, John Wiley and Sons, New York.

12 Schreiber and Shriner, *J. Am. Chem. Soc.*, 1934, **56**, 115.

§ Mayer gives m.p. 161°, solubility in water 0.12 g per 100 cc.

ble in benzene and chloroform. The solid product may be kept in the ice chest for at least 2 months without decomposition.

Analysis. Calculated for $C_6H_8N_2SO_3$: C, 38.29; H, 4.28; N, 14.89; S, 17.04. Found: C, 38.08; H, 4.37; N, 14.40; S, 16.84.

Since the elementary analysis would not show the presence of small amounts of sulfanilamide or the corresponding azoxy compound, a series of preparations of the hydroxylamine was evaluated by the dye reduction method given below. Eight preparations which appeared to be of high purity by melting point gave reduction values in 20 mg % solution between 52 to 53.8%.‖ On diazotization and coupling, as used for determining sulfanilamide,[13] first a yellow, then an orange, and in 2-3 minutes a pink color develops which is, however, only 3-4% of the color obtained from an equivalent of sulfanilamide. When a 0.3 mg % solution was treated with acetic anhydride according to the method of Rosenthal and Bauer,[6] 46-50% of the color expected from an equivalent of untreated sulfanilamide was obtained.

To establish further the structure of the hydroxylamine, the monoacetyl derivative was prepared by acetylation in aqueous solution with acetic anhydride. The product p-(N-hydroxyacetamido)benzenesulfonamide separated from 50% alcohol in glistening white plates, melting point 227-229.5 (dec).

Analysis. Calculated for $C_8H_{10}N_2SO_4$: C, 41.73; H, 4.38; N, 12.17; S, 13.92. Found: C, 41.73; H, 4.33; N, 11.85; S, 13.99. No color was produced on diazotization and coupling,[13] and the product gives a blood-red color with ferric chloride, which establishes the position of the acetyl group on the nitrogen.

On oxidation of the hydroxylamine by oxygen in alkaline solution, p,p′-azoxybisbenzenesulfonamide resulted. The product crystallized from 25% dioxane in 95% alcohol as light yellow irregular prisms, melting point 301-2°C (dec)¶

Analysis. Calculated for $C_{12}H_{12}N_4S_2O_5$: C, 40.44; H, 3.40; N, 15.72; S, 17.99. Found: C, 40.56; H, 3.52; N, 15.85; S, 17.95. It is soluble in alkali, but of low solubility in water and the usual organic solvents.

Method of Analysis and Stability. Owing to the extreme instability of the hydroxylamine under certain conditions, some method of determining actual concentrations in solutions as well as of assay-

‖ A preparation of the hydroxylamine made by Dr. Hugo Bauer and kindly sent us gave a reduction value of 51.5%.

13 Bratton and Marshall, *J. Biol. Chem.*, 1939, **128**, 537.

¶ Mayer gives m.p. 300°C.

ing solid preparations is necessary. The reduction of a standard solution of 2-6 dichlorophenolindophenol was utilized for this purpose. The reduction is not stoichiometric and varies with different aromatic hydroxylamines; an empirical calibration with the pure hydroxylamine was used.

A solution of 2-6 dichlorophenolindophenol of a concentration of about 10 mg % is standardized against a solution of pure ferrous ammonium sulfate.[14] An amount of this solution calculated to contain 4 mg of dye and 100 cc of an M/15 phosphate buffer of pH 7.0 are diluted to 200 cc. Distilled water which has been boiled and cooled to expel most of the dissolved air is used in preparation of the above solutions. To determine hydroxylamine in a solution, 2 cc of the solution (containing from 2 to 20 mg%) is added to 20 cc of the dye-buffer-mixture and the resulting color compared in a colorimeter against 20 cc of the mixture to which 2 cc of water has been added. The comparison should be made in 1 or 2 minutes. The percentage reduction of the dye is now calculated and the hydroxylamine content of the unknown solution found by reference to an empirically determined calibration curve.**

The stability of solutions of the hydroxylamine depends apparently on the hydrogen ion concentration of the solution. In acid solution, the hydroxylamine is fairly stable and apparently goes only to the azoxy-compound, in neutral or alkaline solution, decomposition is

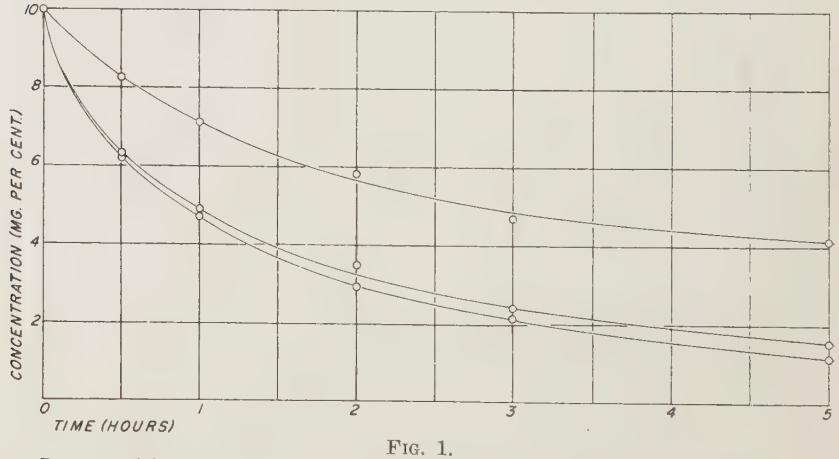

FIG. 1.

Decomposition of solutions of the hydroxylamine in peptone-glucose broth at pH 7.3. Upper curve 23°, middle curve 37°, lower curve 39.3°C.

[14] Lorenz and Arnold, *J. Ind. Eng. Chem.* (anal. ed.), 1938, **10**, 687.

** Reduction values for 20, 12.5, and 9.4 mg % were respectively 52.0, 40.7 and 34.2%.

accelerated and both azoxy-compound and sulfanilamide are formed.

Fig. I shows the rate of decomposition of 10 mg % solutions in broth at 23°, 37° and 39.3°C. The percentage conversion of the hydroxylamine to sulfanilamide after complete decomposition was quite variable (19-42). The precipitate which formed was identified by mixed melting points as the azoxy compound. Higher or lower concentrations than the above of the hydroxylamine (1 to 100 mg %) appeared to decompose at about the same relative rate, and the presence of an inoculum of β-hemolytic streptococci in the broth did not change the rate of disappearance of the hydroxylamine.

Even if 50 mg % of the hydroxylamine is added to dog's blood, none can be recovered in a trichloroacetic acid filtrate using the dye. When 5 mg % are added to rabbit's or dog's blood at 39°C, after 5 minutes, only 40% of the hydroxylamine is converted to sulfanilamide, in 3 hours 75%. In 24 hours dog's blood gave 75 and rabbit's blood 92% conversion.

Injection into Animals. The hydroxylamine is not extremely toxic for animals; no symptoms except methemoglobinemia were noted in dogs on intravenous injection of 20 mg per kg, while in rabbits no symptoms were noted with 30 to 50 mg per kg injected intravenously over 1½ hours. β-phenylhydroxylamine and β-(p-tolyl)-hydroxylamine are much more toxic: the lethal dose for dogs was 20 mg per kg or less. The increased toxicity of these compounds was confirmed on mice injected subcutaneously (the latter 2 hydroxylamines being at least 10 times as toxic as p-hydroxaminobenzenesulfonamide).

When injected into rabbits and dogs, a reaction for an arylamine (presumably sulfanilamide) is obtained in blood and urine. After injections of the hydroxylamine and of an equivalent amount of sulfanilamide in two dogs, the sulfanilamide content of blood and urine was determined. Both experiments gave similar results so that only one is described briefly.

Dog. 11.3 kg. Injected intravenously with 20 mg per kg of the hydroxylamine dissolved in 10 cc of water. Injected intravenously one week later with 18.8 mg per kg of sulfanilamide in 15 cc of water. Table I gives a summary of the results. The first half-hour

TABLE I.
Sulfanilamide Content of Blood and Urine.

Time in min after injection of	Blood concentration, mg %							Urine content, mg	
	5	15	30	60	120	240	24 hr	4 hr	24 hr
Hydroxylamine	3.0	2.5	2.4	2.2	1.9	1.6	0.5	58	130
Sulfanilamide	2.6	2.5	2.4	2.2	2.1	1.7	0.5	44	125

sample of urine after injection of the hydroxylamine probably contained small amounts of this substance (not more than 2-3% of the amount injected) because it gave the acetic anhydride reaction of Rosenthal and Bauer[6] and gave greater reduction of the dye than samples of urines taken before or after this period. This phenomenon was not observed when sulfanilamide was injected.

Bactericidal Action. The relative bactericidal effect of sulfanilamide and the hydroxylamine has been determined at 37° and 39.3° with moderate and large initial concentrations of β-hemolytic streptococci (C 203). These experiments were performed by the test procedure described by one of us[15] for measuring the antibacterial activity of compounds *in vitro* with the following modifications. The peptone-glucose broth used as the test medium contained 2.0% of tryptose (Difco), 0.1% of peptone (Pfanstiehl), sodium chloride 0.5%, glucose 0.2%, and a mixture of phosphates (0.02 molar) to buffer it at pH 7.3. The hydroxylamine was added to broth immediately after seeding with bacteria, and the tubes rapidly brought to test temperature in the water bath. This insured test concentrations of the hydroxylamine within 10% of the calculated value. Table II summarizes the results. The only other information given by the data was that the hydroxylamine is much more inhibitory than sulfanilamide against a large inoculum at 39°. Against the moderate inoculum at this temperature and against both inocula at 37° the inhibitory ratios of the two drugs was similar to the ratios obtained from the bactericidal end points.

Summary. The preparation, properties, stability, and colorimetric

TABLE II.

Bactericidal activity of p-hydroxaminobenzenesulfonamide and sulfanilamide against β-hemolytic streptococcus strain C 203 in peptone-dextrose broth.

Test	Initial bacterial conc. per cc (plate count)	48-hr incubation temperature °C	Minimal bactericidal conc. mg %		Hydroxylamine sulfanilamide activity ratio
			Hydroxylamine	Sulfanilamide	
1	3,000	39.3	4	10	2.5
2	4,500	39.3	6	20	3.3
3	8,500	39.0	6	60	10.0
4	850,000	39.0	>100	400	< 4.0
5	3,000,000	39.3	>100	400	< 4.0
6	4,500,000	39.3	>100	400	< 4.0
7	3,000	37.0	>100	800	< 8.0
8	4,500	37.0	>100	600	< 6.0
9	8,500	37.0	>100	600	< 6.0
10	850,000	37.0	>100	800	< 8.0
11	3,000,000	37.0	>100	1000	<10.0
12	4,500,000	37.0	>100	1000	<10.0

[15] White, *J. Bact.*, 1939, **38**, 549.

analysis of p-hydroxaminobenzenesulfonamide are described. When injected into dogs, this substance appears to be completely converted to sulfanilamide within 5 minutes. *In vitro,* under the conditions of our experiments, it is no more than ten times as active as sulfanilamide.

11071

Vitamin K Deficiency and Prothrombin Levels. Effect of Vitamin K Administration.*

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In using the chick for the assay of vitamin K, certain workers have used the preventive technic,[1] and thus have given an indication of the basal need of the chick for this vitamin. Others have used the curative technic. Ansbacher[2] has used a curative period of 6 hours, and Thayer and coworkers[3] have lengthened the period to 18 hours. With periods longer than this,[4] the basal utilization becomes significant, we believe, and the assay represents a combination of preventive and curative technics.

Both the preventive and the curative technics supply incidental information concerning physiological problems of great importance. Unfortunately, those who have used brief curative periods have not supplied data on the prothrombin level, but have simply used the whole blood clotting time as a measure of deficiency and of response to treatment. It is our present purpose to give part of the data which are still lacking. It is hoped that these data will help to clarify the various assay procedures now in use, and at the same time provide a much needed quantitative correlation between the prothrombin level and the vitamin K supply.

Materials and Methods. White Leghorn chicks, newly hatched,

* Aided by a grant from the John and Mary R. Markle Foundation. Financial assistance was also supplied by the Graduate College, State University of Iowa. The 2-methyl-1,4-naphthoquinone used in these experiments was prepared and supplied through the courtesy of Dr. George H. Coleman and Dr. Donald W. Kaiser, Department of Chemistry.

1 Almquist, H. J., and Stokstad, E. L. R., *J. Nutrition*, 1937, **14**, 235.

2 Ansbacher, S., *J. Nutrition*, 1939, **17**, 303.

3 Thayer, S. A., McKee, R. W., Binkley, S. B., MacCorquodale, D. W., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 194.

4 Dam, H., and Glavind, J., *Biochem. J.*, 1938, **32**, 1018.

were placed on Almquist's diet,[5] modified to contain 2.5% of the salt mixture of Hubbell, Mendel and Wakeman. The salt mixture was also supplemented with 0.2% each of $MnCl_2$, ferric citrate and $AlCl_3$.[6] The ground rice was extracted several days with ether; the fish meal and brewer's yeast were extracted for 10 days in a giant Soxhlet extractor. To prevent bacterial action, the diet was stored at –40°. A control diet, on which normal prothrombin values are based, was similar, but the rice content was decreased sufficiently to permit inclusion of 10% alfalfa meal in the diet. Special care was taken to prevent coprophagy.

Blood for clotting times was obtained by brachial vein incision. Blood (1 cc) for prothrombin determinations was obtained by cardiac puncture, using a 1 cc tuberculin syringe containing 0.2 cc of 1.85% potassium oxalate. The blood was centrifugalized in a special tube and the hematocrit noted. In conducting the 2-stage titration procedure for prothrombin,[7, 8] it was found difficult to defibrinate the plasma completely with thrombin. To make this possible, the plasma was diluted 3-fold with saline as a preliminary measure. In making the prothrombin calculations, a correction was made for this dilution.

Results. The plasma of newly-hatched chicks contains approximately 58 units of prothrombin per cc (Fig. 1). In contrast to this, we have found that the plasma of adult roosters contains approximately 175 units per cc.[9] Work from our laboratory[10, 11] has already shown that the prothrombin level in human infants is likewise about 30% of normal. In both cases the results were obtained with a 2-stage titration technic in which complete conversion of the prothrombin into thrombin is followed by titration of the latter. We believe that the total amount of thrombin which can be formed is the best index of the amount of prothrombin present. The high values obtained in baby chicks by other workers[12] may well be due to the use of prothrombin tests which are based primarily upon prothrombin conversion rate.[11]

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[5] Almquist, H. J., *J. Biol. Chem.*, 1936, **114**, 241.

[6] Hubbell, R. B., Mendel, L. B., and Wakeman, A. J., *J. Nutrition*, 1937, **14**, 273; Wilgus, H. S., Jr., Norris, L. C., and Heuser, G. F., *J. Nutrition*, 1937, **14**, 155.

[7] Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667.

[8] Smith, H. P., Warner, E. D., and Brinkhous, K. M., *J. Exp. Med.*, 1937, **66**, 801.

[9] Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1939, **125**, 296.

[10] Brinkhous, K. M., Smith, H. P., and Warner, E. D., *Am. J. Med. Sci.*, 1937, **193**, 475.

[11] Owen, C. A., Hoffman, G. R., Ziffren, S. E., and Smith, H. P., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 181.

[12] Schønheyder, F., *Am. J. Physiol.*, 1938, **123**, 349.

The 58 unit prothrombin level of newly hatched chicks is an average obtained from the study of many chicks. Inspection of individual protocols indicates that analyses made during the summer months tend to be at least 20% higher than those made in the late fall. It has been suggested by others[13, 3] that the storage of vitamin K in the egg, and the rapidity with which chicks become deficient when on a vitamin K-free diet may vary in different seasons because of differences in diet supplied the laying hens. As far as we are aware, our observation is the first to suggest that the prothrombin level at time of hatching may have a corresponding variation.

When the chicks were placed on the K-deficient diet a gradual fall in the plasma prothrombin level began almost at once (Fig. 1). The fall was not quite so rapid, however, as between the 3rd and 6th days of life. It is evident, nevertheless, that the reserves of vitamin K, carried over from the egg, are not great. The chicks hatched during the summer months maintained their prothrombin levels a day or two longer than those hatched in the late fall, indicating, again, somewhat greater storage of vitamin K in the former.

At about the 7th day of life the clotting time of whole blood becomes prolonged, and at that time the plasma prothrombin level was found to be approximately 30% of normal for chicks of the same age. The variability of clotting time is considerable, however, and the results do not reflect accurately the changes in prothrombin level.

During the next few days the prothrombin level fell slightly more,

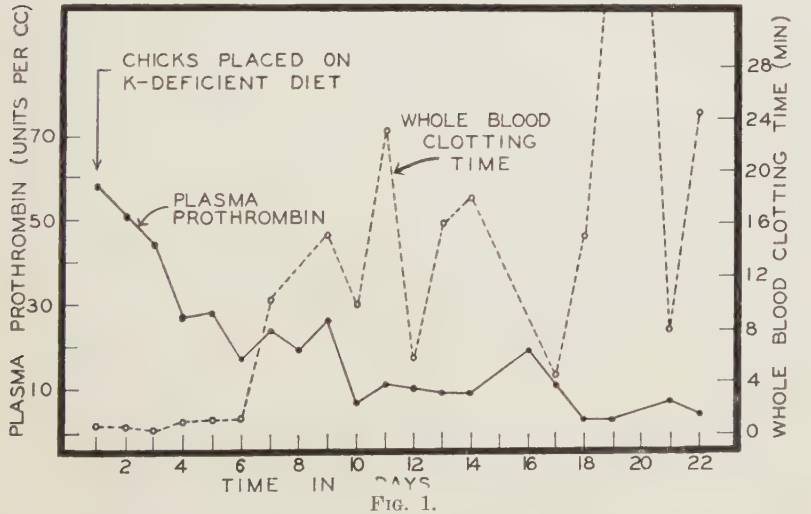

FIG. 1.
Prothrombin Depletion Curve.

[13] Almquist, H. J., and Stokstad, E. L. R., *J. Nutrition*, 1936, **12**, 329.

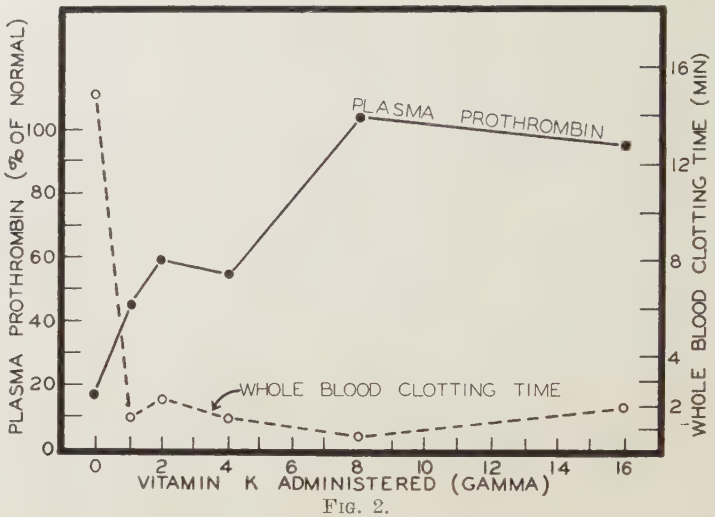

FIG. 2.
Prothrombin Levels. Effect of Vitamin K.

and a number of the chicks showed spontaneous hemorrhages. It is evident that the bleeding level is reached when the prothrombin falls to approximately 10% of the values seen in normal chicks of the same age.

Fig. 2 shows the effect of single doses of vitamin K in effecting cures in deficient chicks. Varying doses of 2-methyl-1,4-naphthoquinone (1.0-16.0 gamma in 0.2 cc corn oil) were placed directly in the crop. After the lapse of 18 hours the clotting time and the prothrombin level were determined. Doses of 8 gamma, or more, uniformly brought the prothrombin level almost completely to normal in this length of time. Smaller doses had progressively less effect, though a definite rise was still evident on administration of as little as 1.0 gamma of the compound.

The question at once arises as to whether an 18-hour period is sufficiently long to permit the maximum effect of the vitamin to become manifest. Our data on this point are still incomplete, but evidence indicates that 18 hours produces almost as much effect as 42 hours, even in cases where the dose is inadequate to effect complete recovery.

When the recovery period is shortened to 6 hours, recovery was again found to be practically complete in case the dose was 16 gamma or more. With smaller doses this is not the case. We have performed a number of experiments at the 8 gamma level, and have found that after 6 hours the prothrombin recovery was only 50% complete.

The whole blood clotting time, prolonged in the deficient chicks prior to treatment, returned to normal after the chicks were treated with 1 gamma of the vitamin. The prothrombin level was still less than one-half of its normal value, however. It is thus evident that those vitamin K assay technics which are based upon whole blood clotting time do not indicate the amount of vitamin needed to effect a complete cure. At most, they indicate approximately the amount of the vitamin needed to bring the prothrombin above the 30% level.

The use of the 6-hour curative period in assay work introduces a second factor which has not been recognized, *i. e.,* the fact that adequate time is not allowed for the maximum action of the vitamin. If the 6-hour principle is combined with the use of whole blood clotting time as an index of deficiency, it is evident that the two variables tend to neutralize each other, though to an unpredictable degree.

Much of the assay work to date has been done for the purpose of comparing two or more products of unknown potency. In dealing in this way with relative values, it is probable that any one of the various technics reported would give comparable data. Any effort, however, to establish absolute biological units should take into account the facts which we have outlined.

The fact that large doses effect recovery more rapidly than smaller ones has important implications for the clinician. It is often a matter of great importance that the bleeding tendency seen in vitamin K deficiency be eliminated with minimum delay. When the liver is impaired, or the body has for other reasons a decreased ability to form prothrombin, it is possible that large doses of vitamin K will permit more effective use of whatever capacity may remain for the production of prothrombin. This concept is consistent with the fact that some patients require far more vitamin K than others.

Summary. A detailed study was made concerning the rate at which the plasma prothrombin level falls when newly hatched chicks are placed on a vitamin K-deficient diet. The whole blood clotting time becomes prolonged when the prothrombin level falls to about 30% of the normal level for chicks of the same age. Hemorrhages make their appearance when the prothrombin is approximately 10% of normal.

It was also shown that large doses of vitamin K correct the plasma prothrombin deficit almost completely within 6 hours. Somewhat smaller doses effect partial recovery in 6 hours, and almost complete recovery in 18 hours.

Questions of assay technic and of therapeutic needs are discussed.

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Effect of Venous Pressure on Volume Pulsation.

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In a previous study it was noted that, with ligation of the main venous outlet from the lower extremity of the dog, a marked reduction in peripheral pulse volume occurred.[1] The present observations were made to throw some light on the mechanism of these changes.

In 14 dogs the volume of pulsations in the hind foot was determined by adapting a specially constructed cellulose-acetate cup to the sensitive plethysmograph of Turner.[2] All observations were conducted under ether anesthesia and the operative procedure, consisting of isolation and ligation with silk of the femoral vein at the level of Poupart's ligament, was performed aseptically. Direct venous pressure determinations[3] were made in the saphenous vein at heart level before and after the ligation during the continuous determination of pulse volume. All observations were conducted under controlled atmospheric conditions, temperature 75°F and humidity 50%.

Observations were made on 2 groups of dogs: (1) 6 animals with intact sympathetic innervation, and (2) 8 animals on which resection of the ipsolateral lumbar sympathetic ganglia and intervening chain had been performed 24 hours previously.

Results. The results are graphically represented in Fig. 1. It may be observed that the rises in venous pressure in the femoral vein following ligation were 13.9 and 10.5 times the normal value in the non-sympathectomized and sympathectomized animals, respectively. The decreases in volume of pulsations following the ligations were 51.5% and 58.2%, respectively. The results obtained in each dog were essentially the same as the mean values, except in degree. It may also be observed that although the reactions in the sympathectomized and non-sympathectomized animals were essentially the same, the pulse volumes both before and after ligation were slightly lower in the sympathectomized than in the non-sympathectomized dogs,

[1] DeBakey, M., Burch, G., and Ochsner, A., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 585.

[2] Turner, R. H., *J. Clin. Invest.*, 1937, **16**, 777.

[3] Burch, G. E., and Sodeman, W. A., *J. Clin. Invest.*, 1939, **18**, 31.

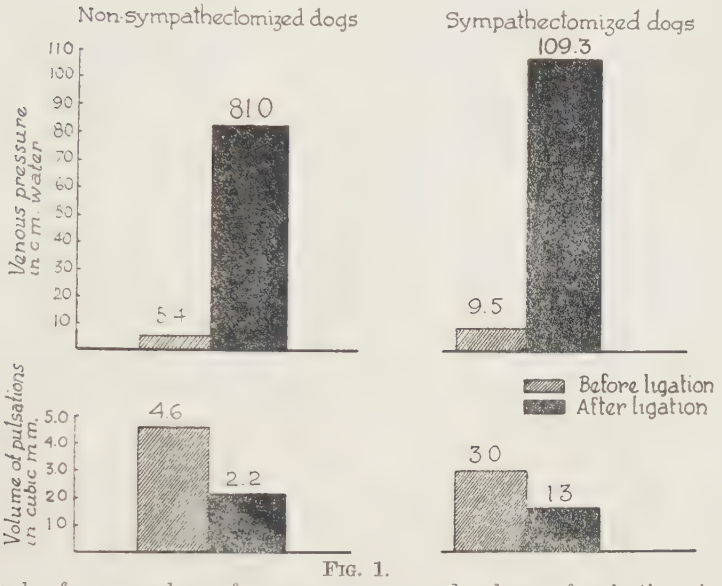

FIG. 1.

Graph of mean values of venous pressure and volume of pulsations in non-sympathectomized and sympathectomized dogs before and after ligation.

and that the corresponding venous pressures were slightly higher in the former.

There are at least 3 possible explanations for the reduction in volume of pulsations following venous ligation: (1) vasoconstrictor reflexes, (2) contraction of smooth muscle, for as demonstrated by Bayliss[4] smooth muscle of vessels, as elsewhere, reacts to a stretching force by contraction, and (3) the reduction in the elastic properties of the vessel produced by mechanical distension. The importance of the factor of vasoconstrictor reflexes transmitted by sympathetic pathways may be excluded by the data already given. Although vasoconstrictor reflexes by way of the sympathetics may have some slight influence, they cannot be considered the significant factor because the reactions in sympathectomized and non-sympathectomized dogs are qualitatively the same.

In an attempt to evaluate the remaining two factors the following experiment was performed. Sections of the dog's femoral artery 5.0 cm in length were removed from hind legs which had been refrigerated at approximately 0°C for 8 days. These were placed in a specially constructed plethysmographic cup and arranged as diagrammed in Fig. 2. The system was filled with normal saline, as indicated in Fig. 2, and a constant pressure of 108 cm of water maintained in the supply bottle. A mechanical interrupter was

[4] Bayliss, W. M., *J. Physiol.*, 1902, **28**, 220.

placed on the rubber tubing between the supply bottle and the vessel to produce a pulsatile flow at a rate of approximately 70/min. Both the collecting bottle and the supply bottle were simultaneously elevated from 0 to 70 cm in 10 cm increments. Thus, pulse pressure was maintained at a constant level throughout the entire procedure, but the initial and final tensions on the artery with each increment were increased. The results are shown in the curve of Fig. 3, which represents the mean values for the 6 arteries. The values for each vessel gave a curve similar to the composite curve for the 6 vessels. It may be observed from the curve that, as the tension within the vessel was increased (the pulse pressure remaining constant), the pulse volume decreased, the magnitude of the change diminishing with the higher tensions.

Whereas in the live animal vasoconstrictor influence as a result of increased intravascular tension may be operative[4, 5] and may be a contributing factor in the response to increased venous pressure, it is not essential to the phenomenon, as demonstrated by the persistence of the reaction following refrigeration. By means of refrigeration for a period of 8 days the smooth muscle in these vessels may be considered no longer reactive.

The third possible explanation for the reduction in pulse volume, the reduction in the elastic properties of the vessel produced by mechanical distension, is strongly supported by the results represented in Fig. 3. The curve is, in essence, one of vessel elasticity in which nervous and myogenic influences have been excluded. The

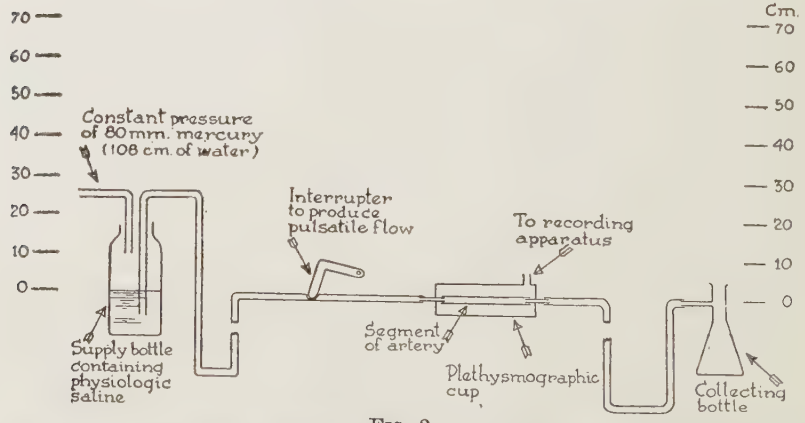

FIG. 2.
Diagram of experimental procedure used in study of isolated refrigerated vessels.

[5] Sodeman, W. A., Burch, G. E., and Turner, R. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 259.

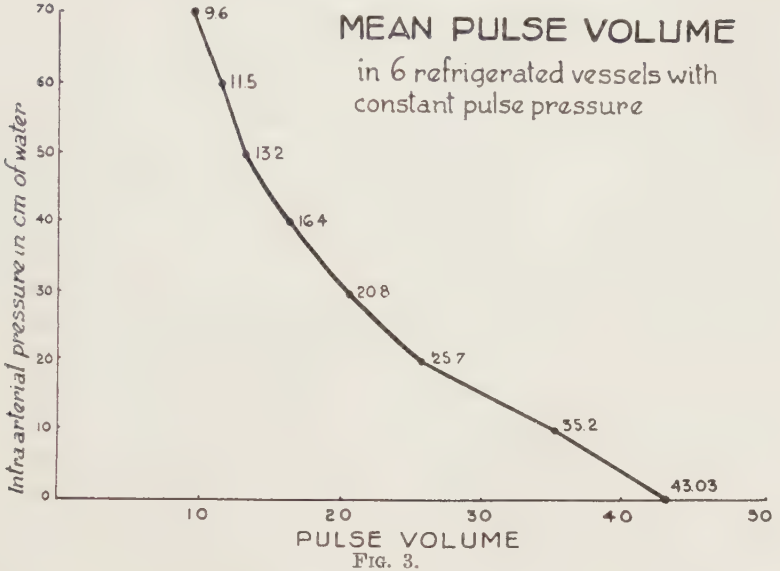

FIG. 3.
Graph of mean pulse volume in 6 refrigerated vessels.

curve is similar to those obtained by Roy[6] on the elastic properties of the arterial wall by a somewhat different technic, and further confirms this explanation.

Although the results of the experiment on the refrigerated vessels cannot be used as direct proof for the possible explanation that the diminution in volume of pulsations, as obtained in the first experiment (Fig. 1) is due to a reduction in the elastic properties of the vessels produced by distension, the fact that such a reaction does occur in other portions of the vascular tree is significant. It suggests that a similar reaction may occur in the more peripheral vessels.

[6] Roy, C. S., *J. Physiol.*, 1880-82, **3**, 125.

AUTHORS' INDEX

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SUBJECT INDEX

(The numerals indicate the page.)